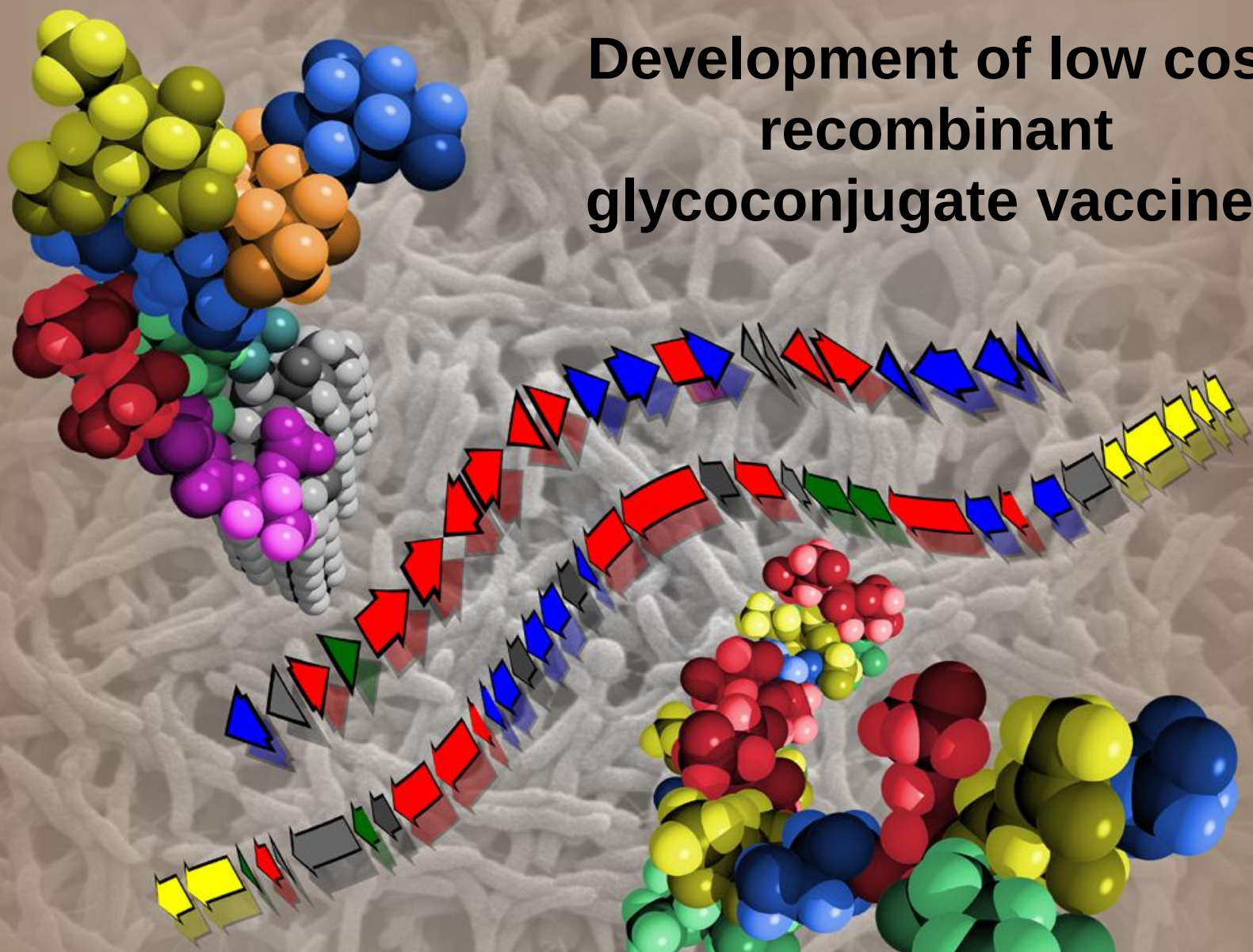
Development of low cost recombinant glycoconjugate vaccines



Brendan Wren
London School of Hygiene & Tropical Medicine

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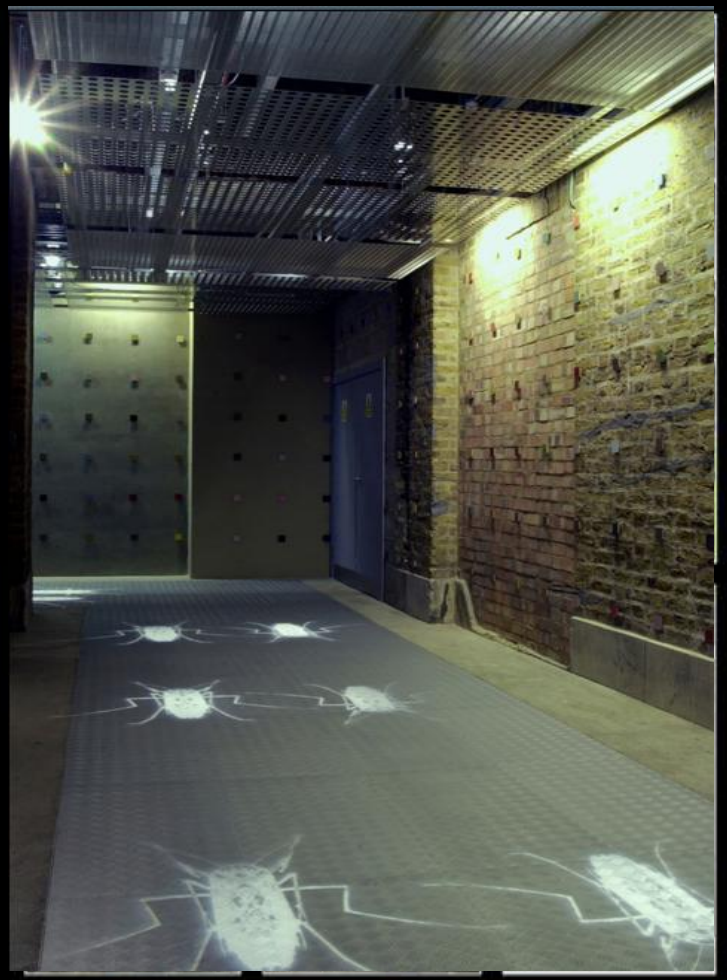
AND TROPICAL MEDICINE



Protein glycosylation

More than 80% of human proteins are modified by addition of sugar structures (glycoproteins, either *O*- or *N*-linked)

Glycoproteins are involved in many biological processes ranging from conception to death

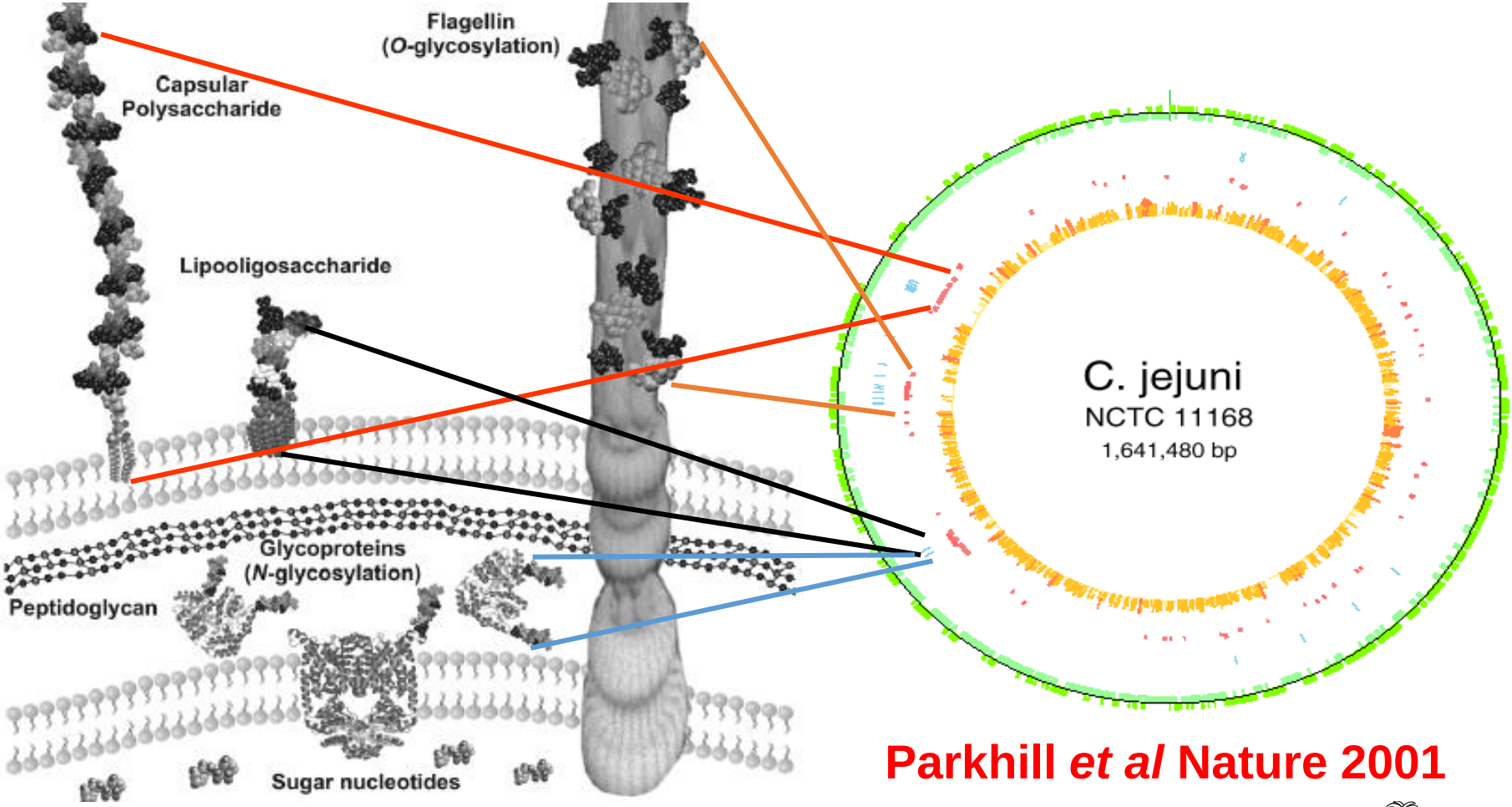
Glycoproteins are complex and difficult to study in eukaryotes, but can be found in bacteria

Glycocode poorly understood

In contrast to the cloning revolution for DNA and proteins, glycoproteins have escaped biotechnological applications

Glycostructures - from genome project to structure & function

Campylobacter jejuni a hyperglycaemic bug >8% genome encode glycostructures



Parkhill *et al* Nature 2001

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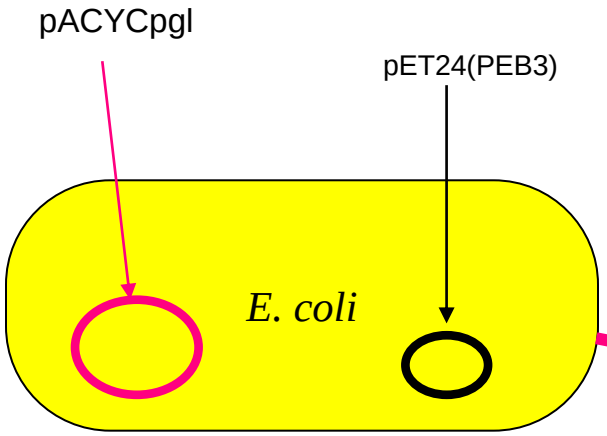
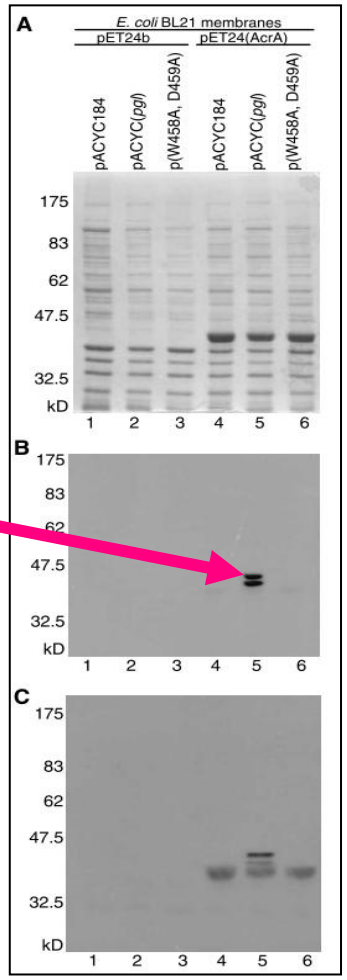
Functional transfer of *Campylobacter jejuni pgl* locus in *E. coli*

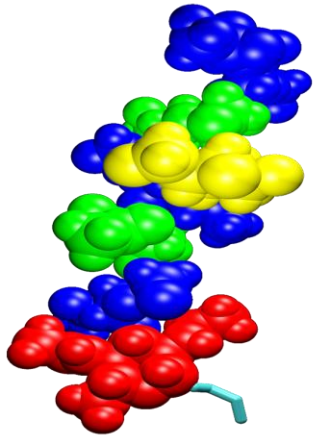




Coomassie

anti-glycan

SBA lectin



System can be used to express glycan in *E. coli*

Wacker *et al. Science* (2002)

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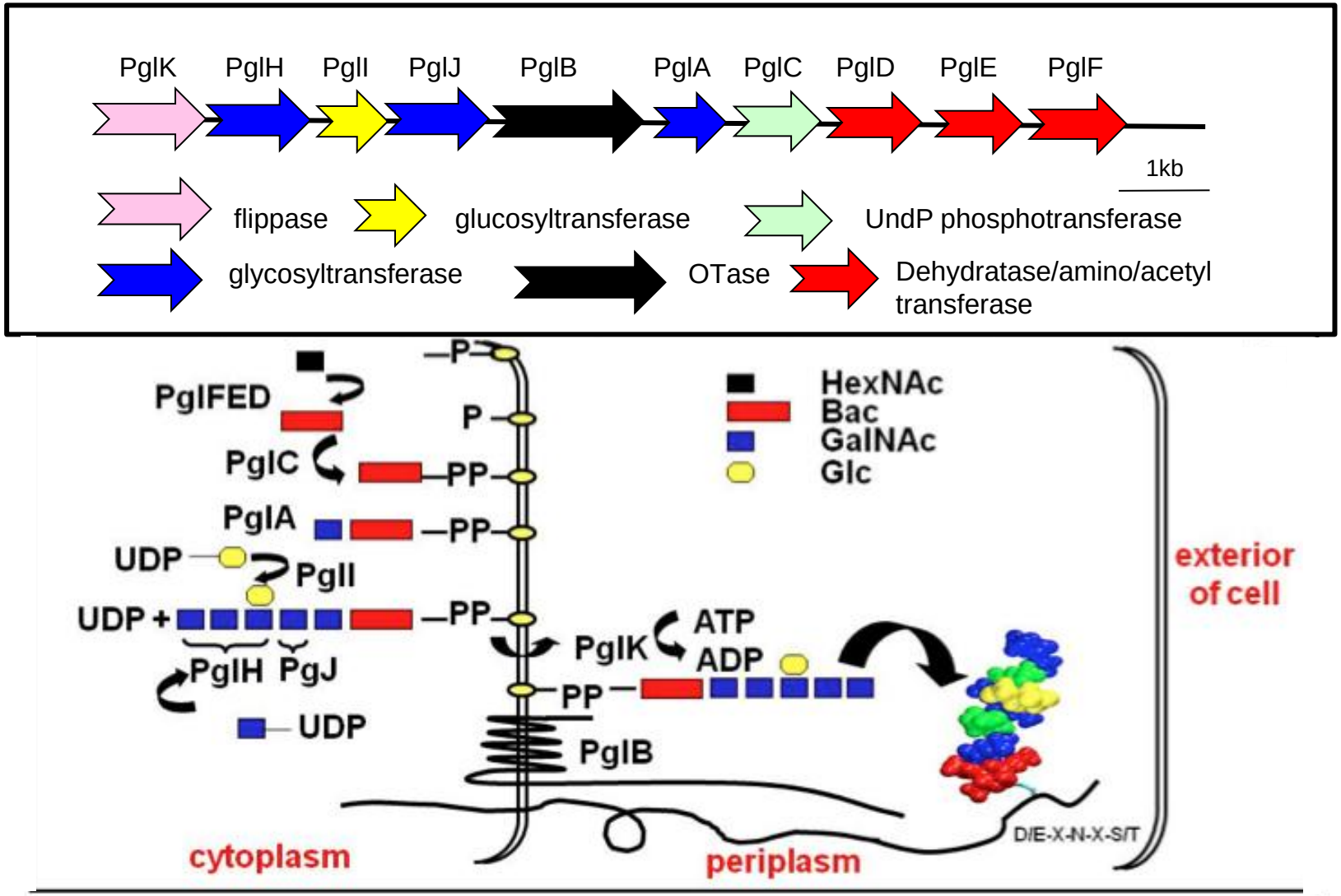
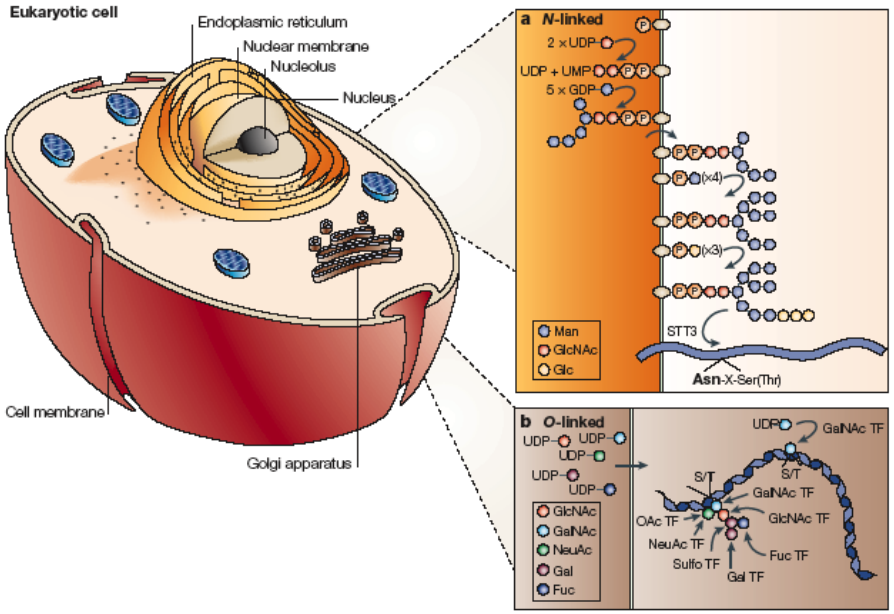
Biosynthesis of *N*-linked glycoproteins in *Campylobacter*



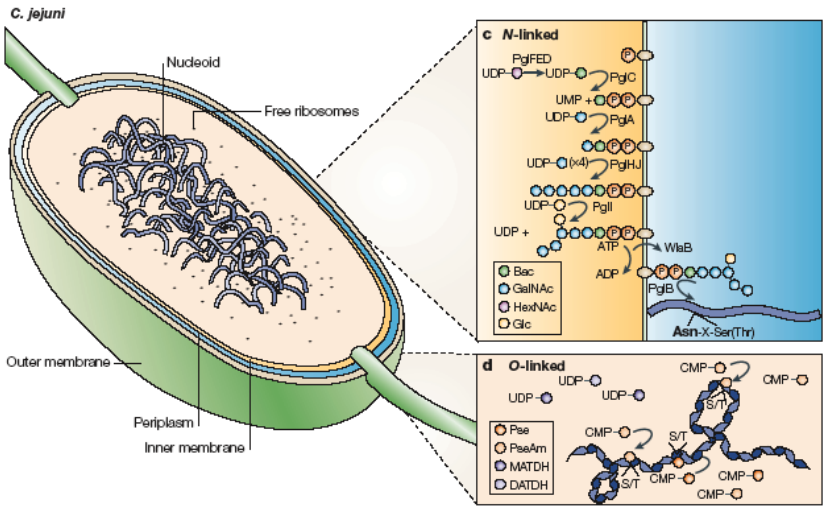
Linton *et al.* Mol Micro 2005

Comparison of bacterial and eukaryotic glycosylation systems



Eukaryotic

Campylobacter



Szymanski & Wren
Nature Rev Micro 2005

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A new era for glycoengineering in bacteria

But how and where to apply this new potential technology?

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Glycoconjugate-based vaccines

Polysaccharide-based vaccines produce a T-cell independent immune response with IgM that opsonises bacteria.

To convert to a more favourable T-cell dependent response polysaccharides are often conjugated to proteins

Examples of successful human glycoconjugate vaccines

1. ***Haemophilus influenzae***
2. ***Neisseria meningitidis*** **(except type B)**
3. ***Streptococcus pneumoniae*** **(some serotypes)**

Long lasting immunity & suitable for infants and elderly
WHO recommend vaccines to be glycoconjugated

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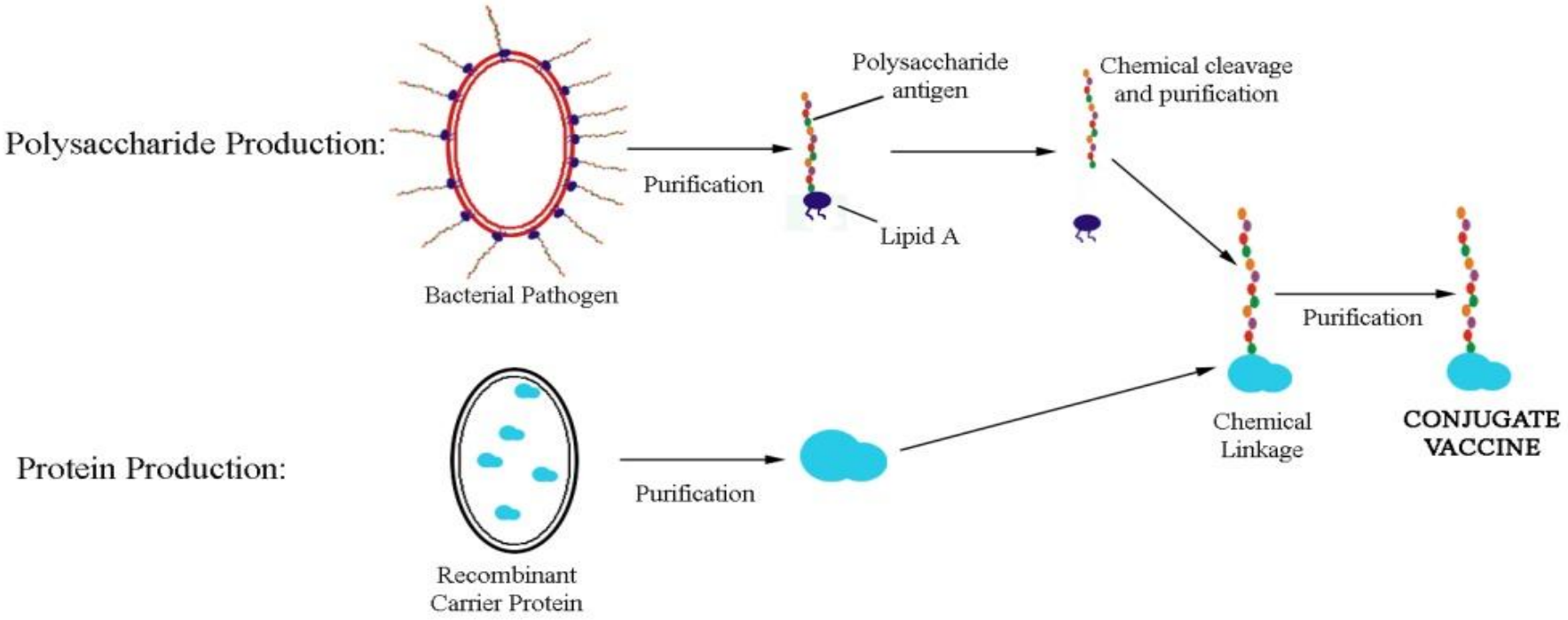
Traditional chemical conjugation



Multistep expensive procedure > 300 quality control steps
Product often heterologous
Expensive

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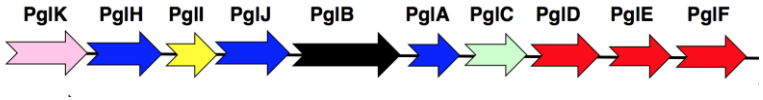
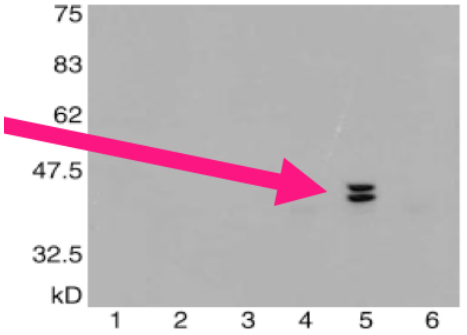
The genesis of bacterial glycoengineering

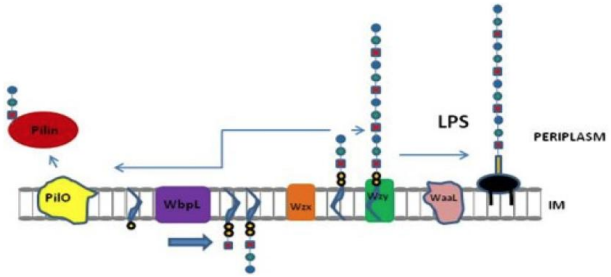
1. **Discovery of *Campylobacter N*-linked glycosylation system**
   (Parkhill *et al.* Nature 2001)



2. **Functional transfer of glycosylation system into *E. coli***
   (Wacker *et al.* Science 2002)



3. **Coupling of capsules and O-antigen to proteins in *E. coli***
   (Feldman *et al*. PNAS 2005)



New processes

- **Glycan Expression Technology (GET)**
- **Protein Glycan Coupling Technology (PGCT)**
- **Glycan Seeking Technology (GST)**

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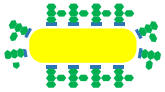
Glycan Expression Technology (GET)

Glycan synthesis genes

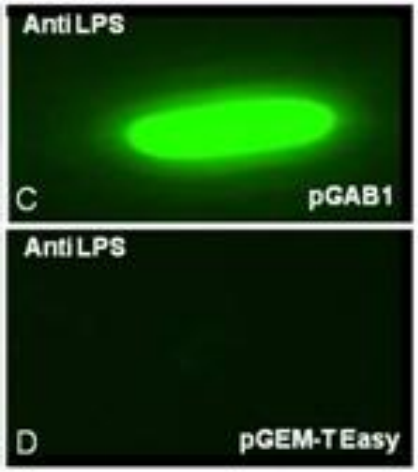
Step 1: Genetic cluster encoding sugar structure is cloned into a safe laboratory strain of *E. coli*

Step 2: Express foreign sugar structure

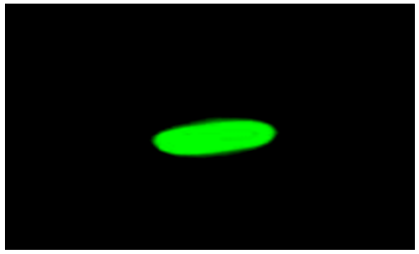
Engineered bacterial strain



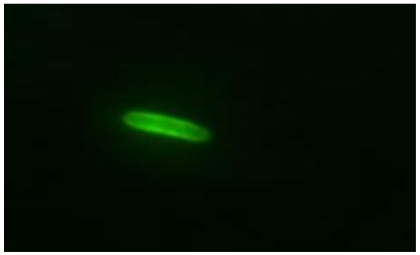
Control bacterial strain



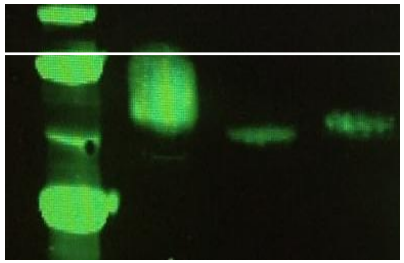
Green glow demonstrates coating of the cell with the new sugar structure



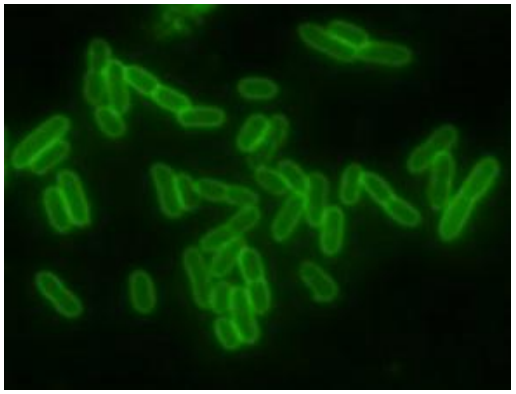
Francisella tularensis O antigen



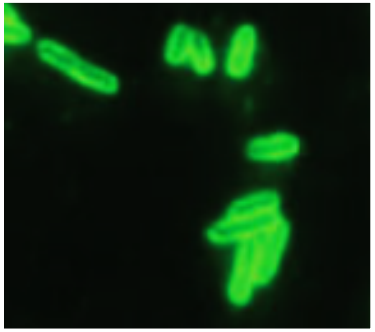
Burkholderia pseudomallei O antigen



Actinobacillus pleuropneumoniae NGT



Streptococcus pneumoniae serotype 8



Streptococcus pneumoniae serotype 4

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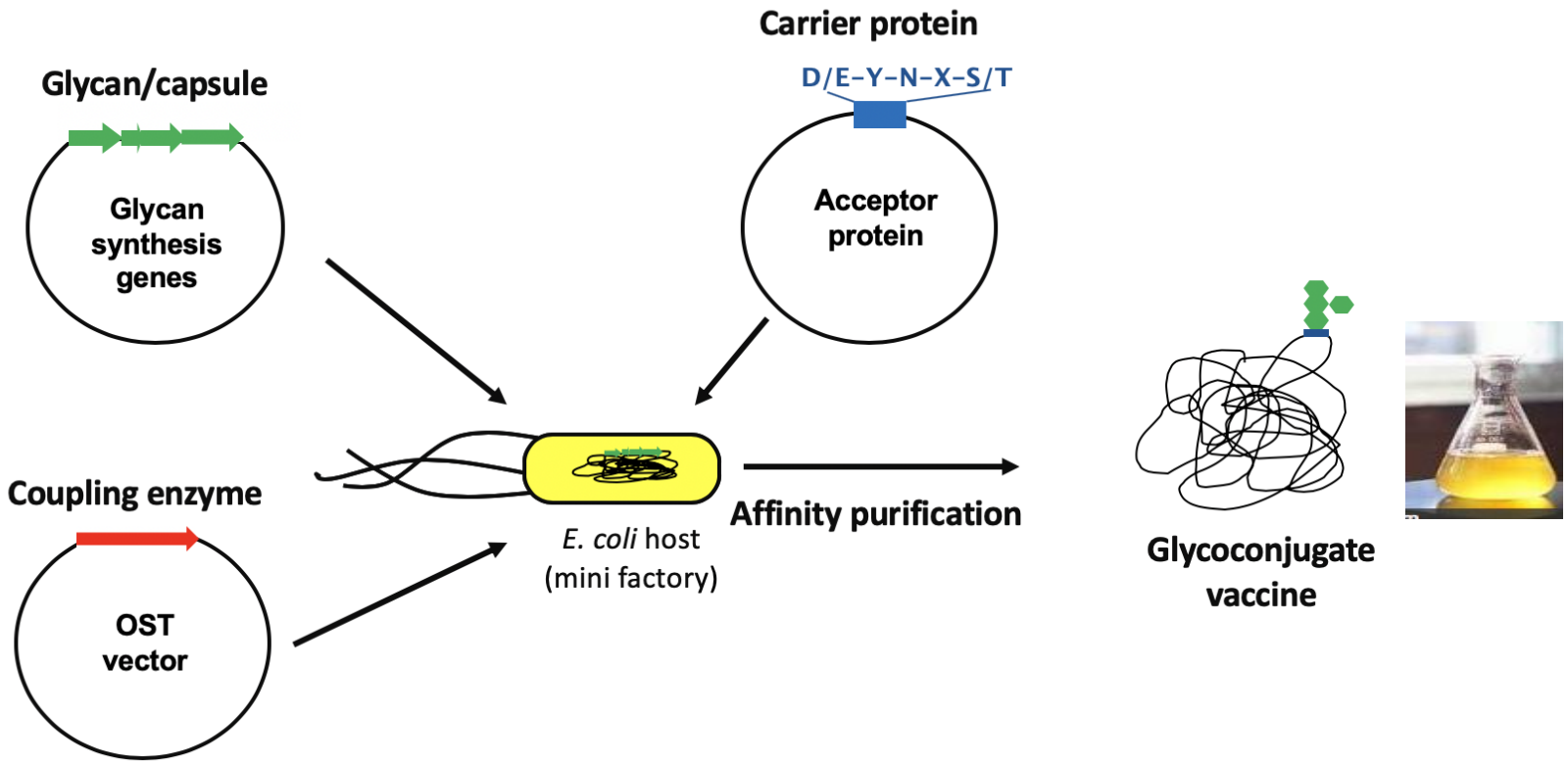
Protein Glycan Coupling Technology PGCT

PGCT allows the bioconjugation of selected glycans to chosen acceptor proteins



Recombinant approach in *E. coli* - one step purification procedure

Flexibility of mixing & matching of protein/glycan combinations

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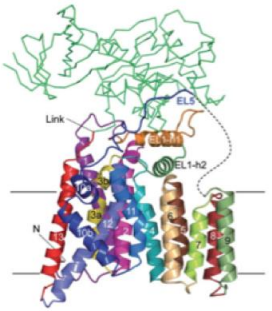
Making an inexpensive recombinant glycoconjugate vaccine in three easy steps

1. **Dial up target protein with glycotags and target glycan & DNA synthesise**

2. **Add DNA encoding protein and glycan to *E. coli* cells expressing coupling enzyme on chromosome**



3. **Grow *E. coli* and purify vaccine from column**

Simple process – *E. coli* is a mini factory

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Current recombinant glycoconjugate vaccines

1. New vaccines

***Eg Francisella tularensis, Burkholderia pseudomallei, Coxiella burnetii, Clostridium difficile, Brucella species, Shigella species* and Traveller's diarrhea vaccine**

2. Improving existing glycoconjugate vaccines

***Eg Streptococcus pneumoniae* (£2 billion per year)**

3. New markets

***Eg* Poultry and pig glycoconjugate vaccines**

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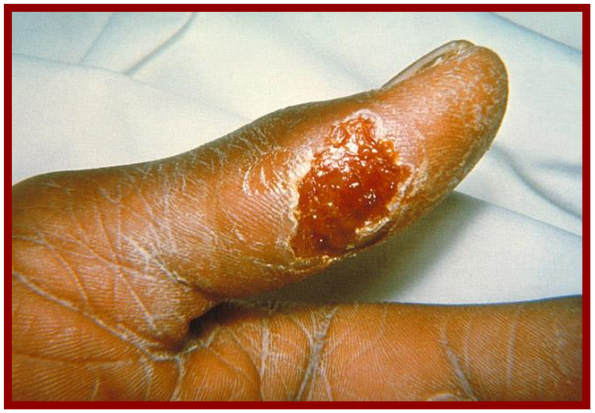
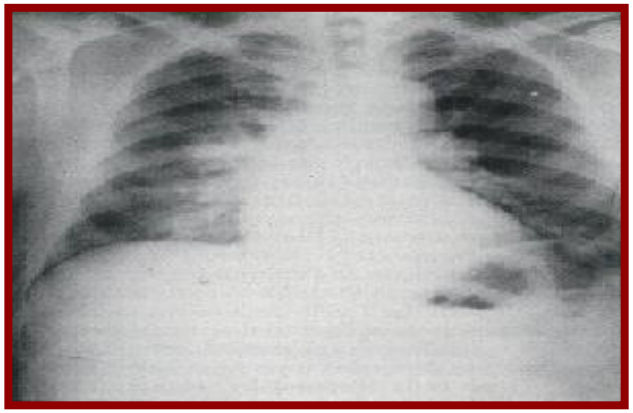
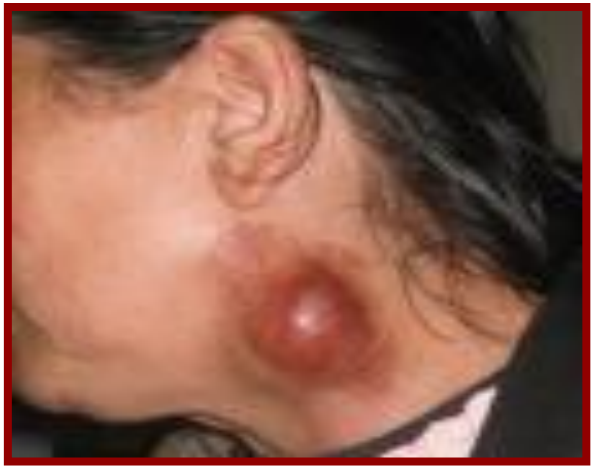
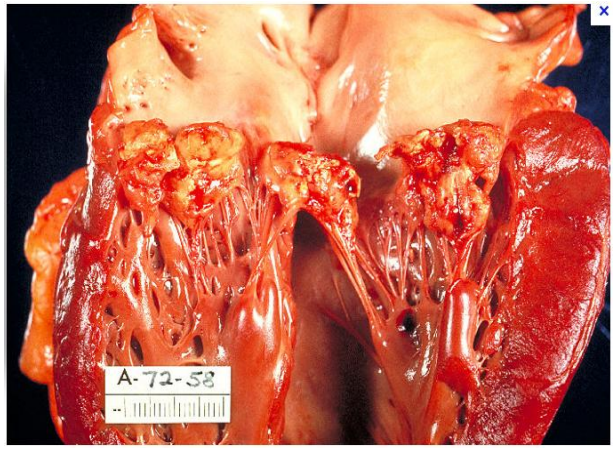
Francisella tularensis
lethal disease – no current vaccine

Intracellular pathogen – low infectious dose of just 10 bacteria









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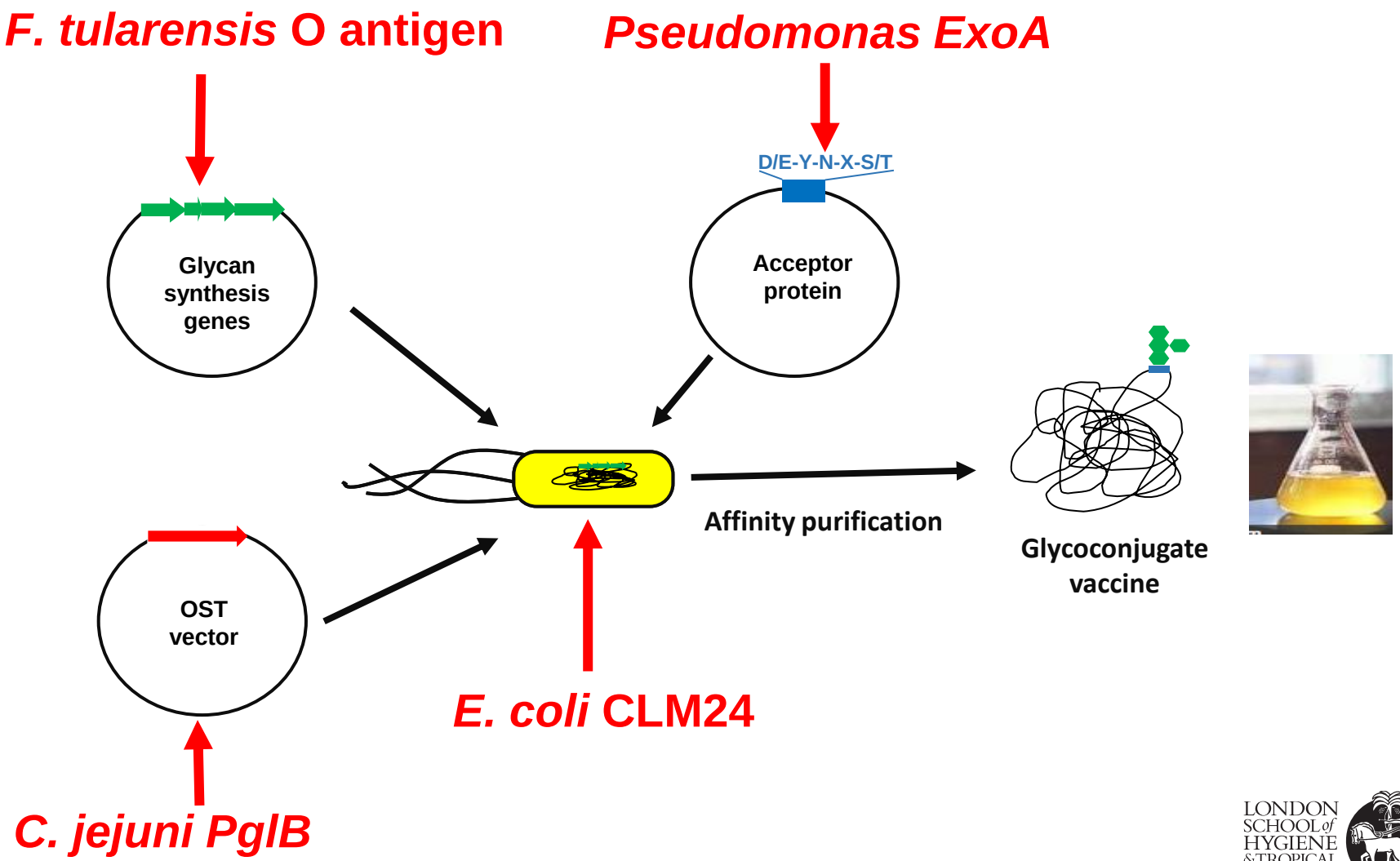
F. tularensis glycoconjugate vaccine design



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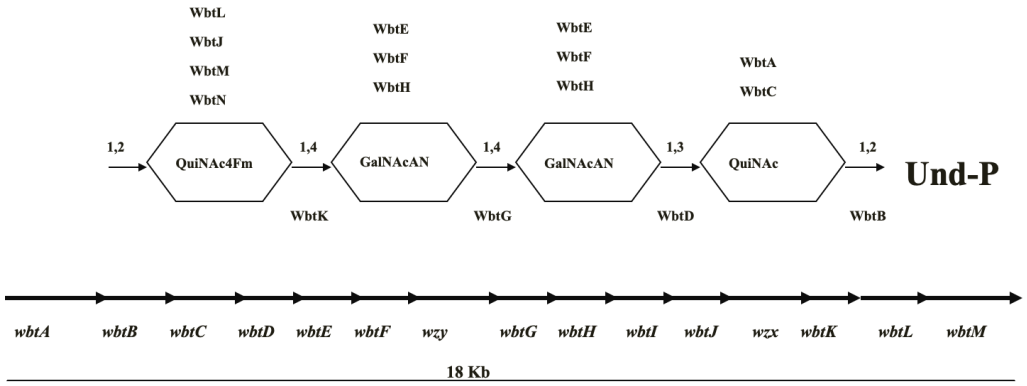
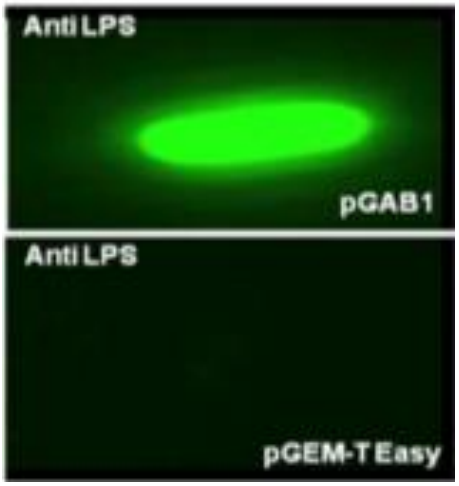
Glycan expression technology
select and express glycan locus in *E. coli*



***Francisella* LPS has terminal QuiNAc**



Confirmed LPS expression in *E. coli*

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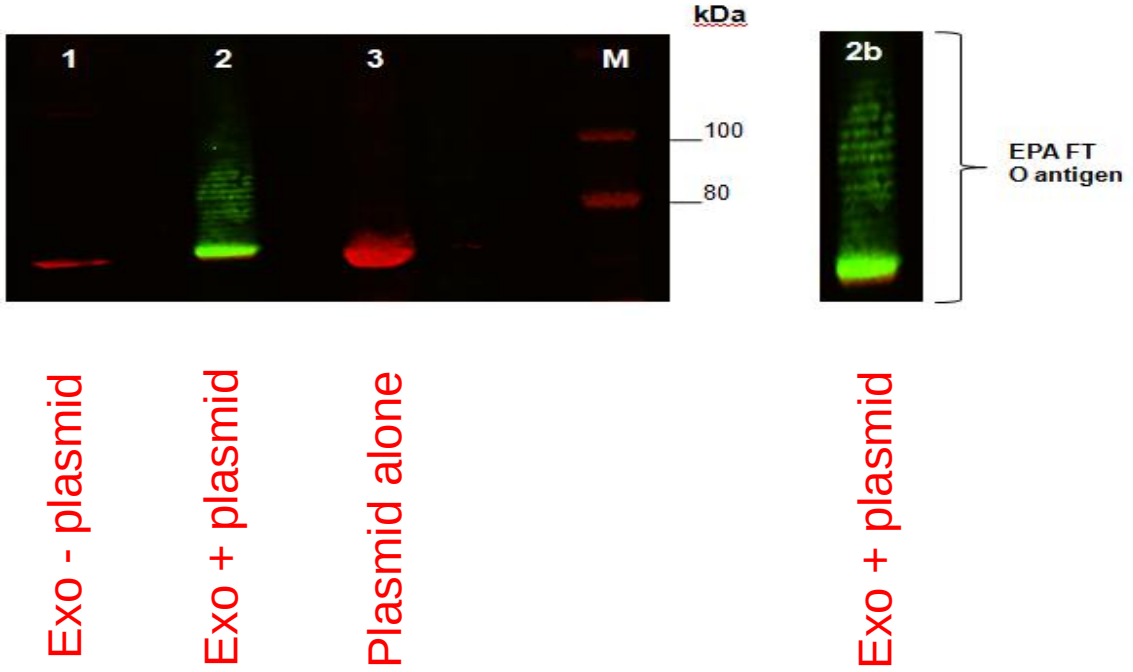
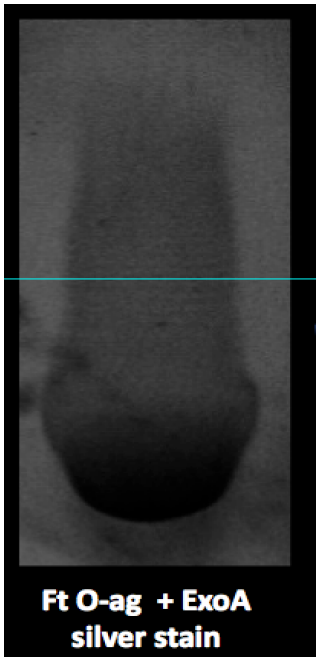
PGCT - add protein carrier and CjPglB coupling enzyme

Red ab stain of ExoA

Green ab stain of *Francisella* O-antigen (Mab FB11)





Yield 100 mg per 10 L *E. coli*

Cuccui *et al* Open Biol 2013

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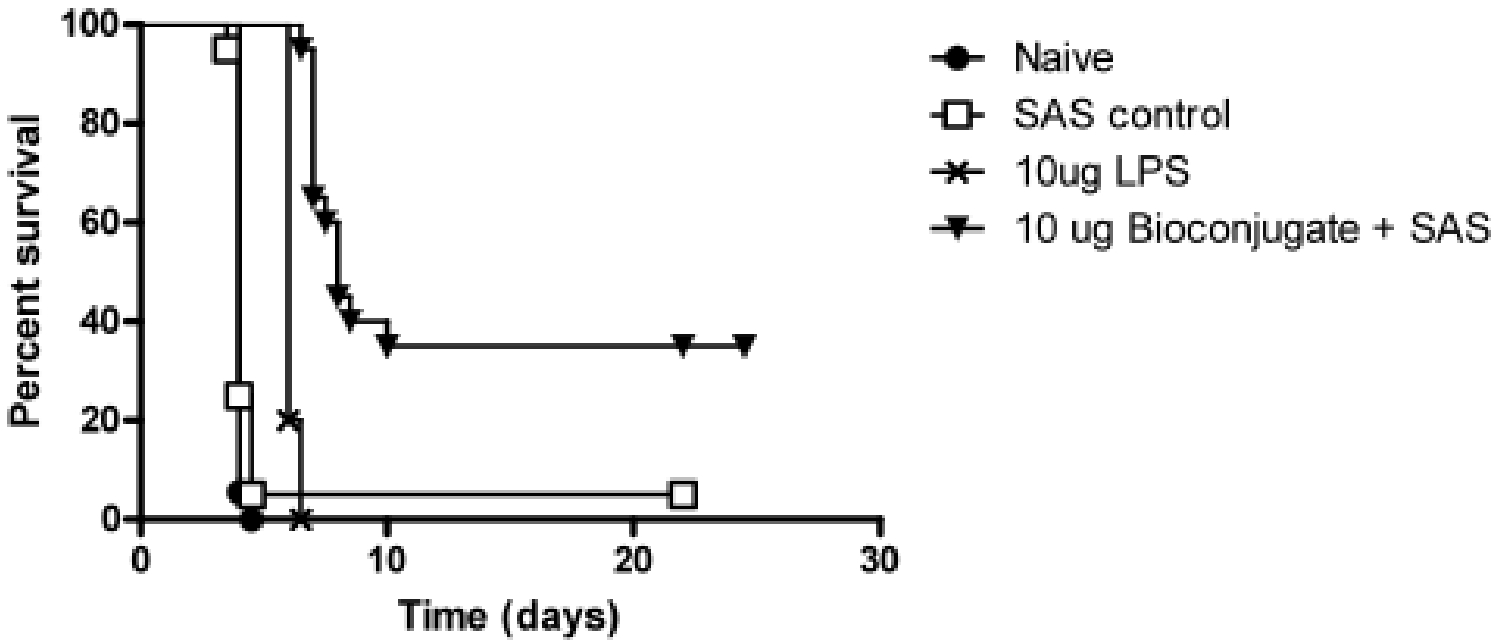
Francisella tulerensis LPS coupled to exotoxin A

Tested in mice, first attempt - best vaccine to date



Confirmed protection & Th1-dependent response

Cuccui *et al* Open Biol 2013

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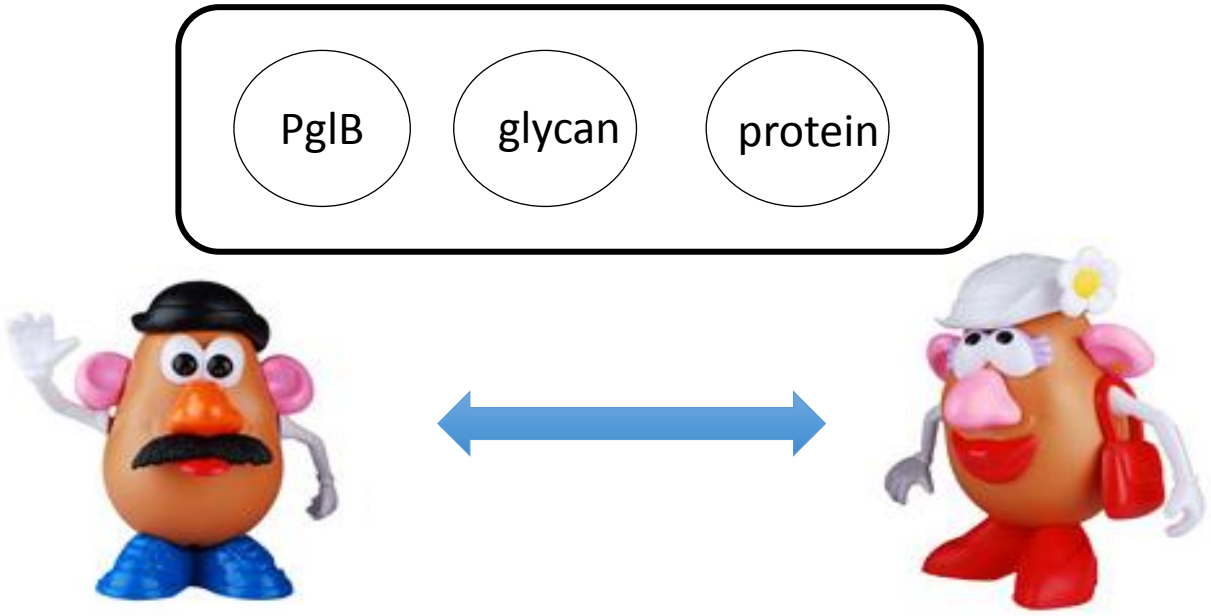
Second & third generation PGCT glycoconjugate vaccines



1) Alter Exotoxin A carrier protein to be more heavily glycosylated

2) Swap carrier protein to a native *Francisella* protein to provide dual protection against glycan and protein

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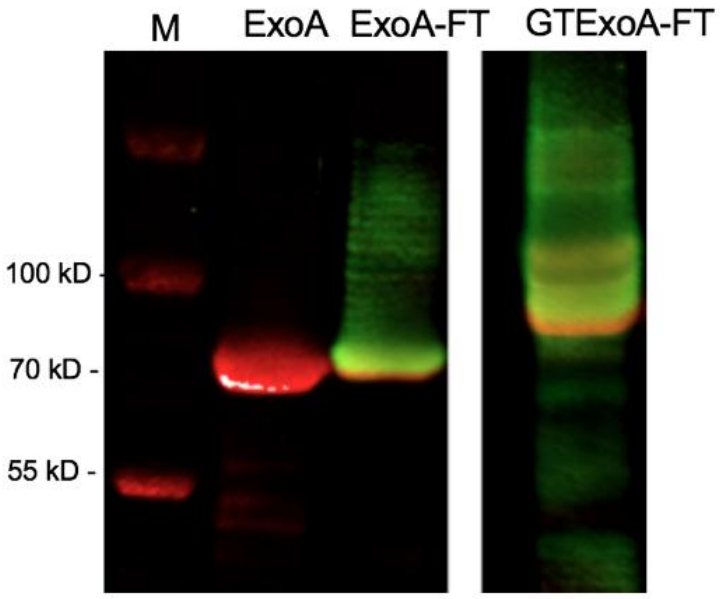
Second generation
Heavily glycosylated ExoA with glycotags

Red ab stain of ExoA

Green ab stain of *Francisella* O-antigen (Mab FB11)



GTExoA+++ best protection yet in both mice and rat infection models

Whelan et al., Journal of Immunology 2018

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Third generation - “Double hit” approach
Francisella carrier proteins

Candidates

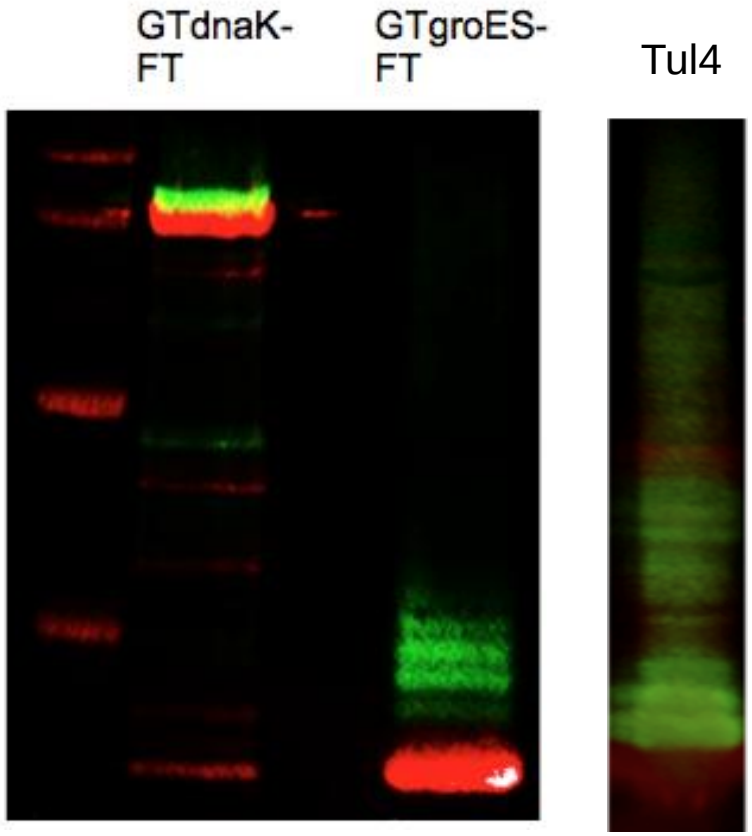
GroEl

GroES

DnaK

IglB

Tul4



Samples purified and ready to be tested in mice and rats

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Burkholderia pseudomallei
lethal disease – no current vaccine

- Gram-negative facultative motile rod bacterium
- Environmental saprophyte endemic in SE Asia and N Australia.
- Intracellular pathogen, causative agent of melioidosis.
- Infection can occur through contamination of wounds or inhalation.
- Acute septicaemia. 40-80 % mortality despite therapy.
- Chronic/Latency up to 62 years reported.
- No vaccine available.
- Infectious dose = 10
- Profoundly antibiotic resistant
- Select Agent Tier 1

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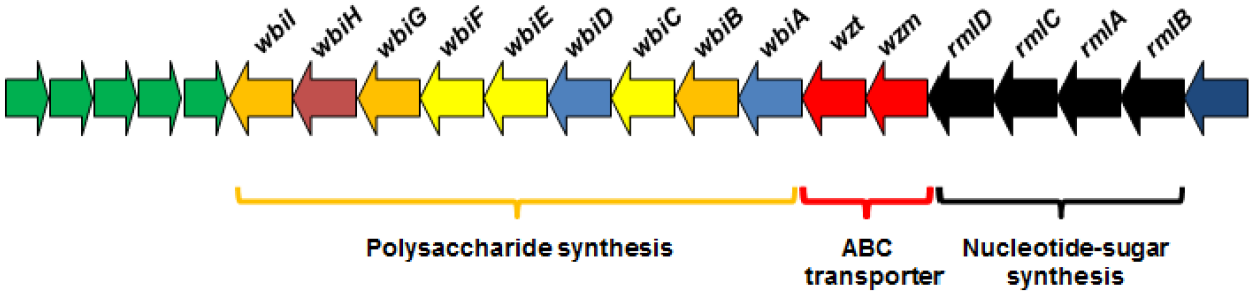
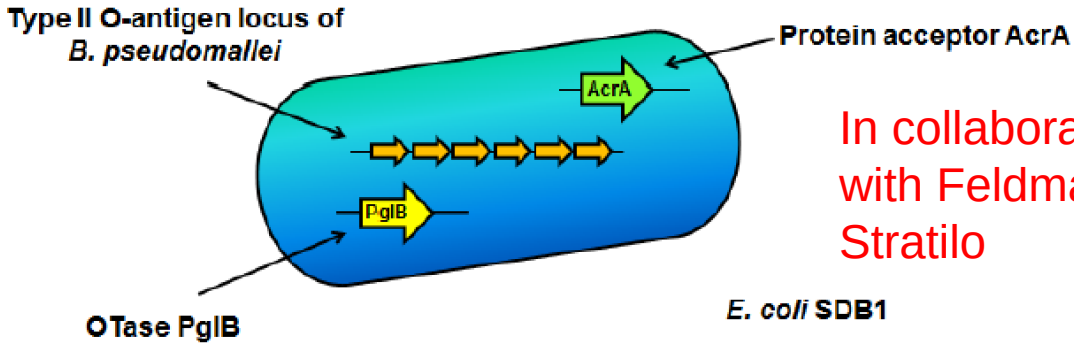
Recombinant *Burkholderia pseudomallei* glycoconjugate vaccine design

Organization of *B. pseudomallei* K96243 O-antigen polysaccharide (II) locus



II O-PS (LPS)	heteropolymer (disaccharide)	-3)- **β-D-glucopyranose**-(1-3)-**6-deoxy-α-L-talopyranose**-(1- 33% of L-6dTalp = 4-o-acetylated, 2-o-methylated. 67% = 2-o-acetylated



In collaboration with Feldman & Stratilo

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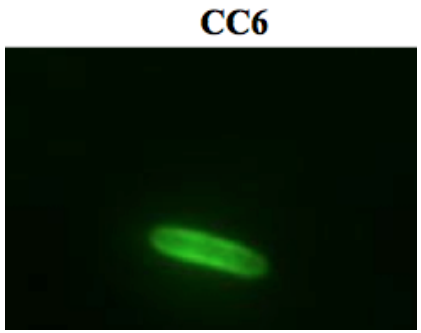
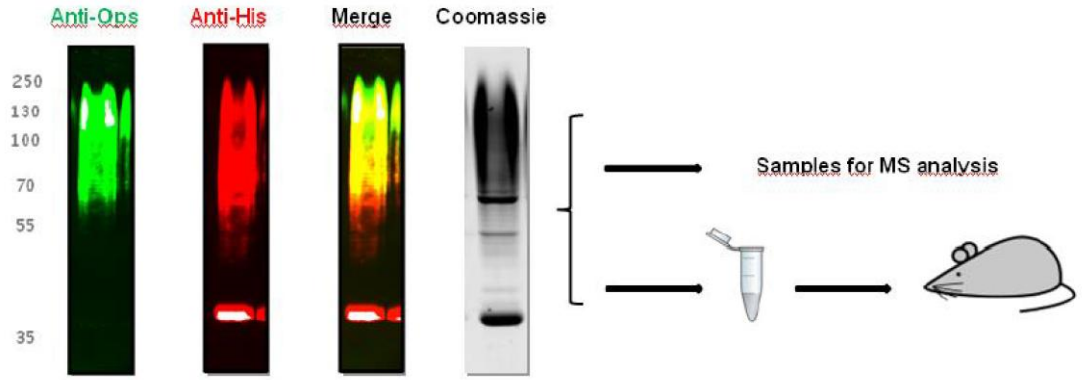
Purification and testing of *B. pseudomallei* vaccine

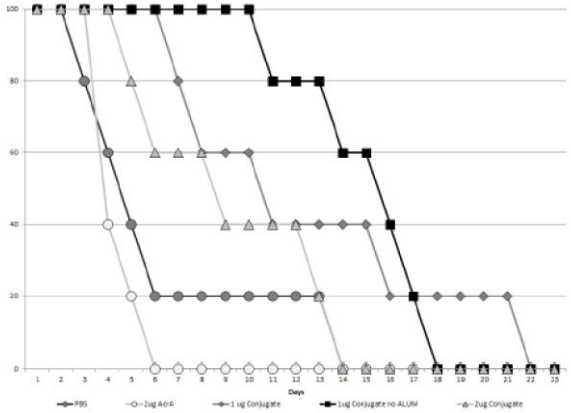




LPS expressed in *E. coli*

Coupled to AcrA and purified

In collaboration with Feldman & Stratilo



Protection in mice

***Burkholderia* carrier protein candidates**

LolC

HcpI

Others from Thai patient study?

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Current recombinant glycoconjugate vaccines

1. New vaccines

***Eg Francisella tularensis, Burkholderia pseudomallei, Coxiella burnetii, Clostridium difficile, Brucella species, Shigella species* and Traveller's diarrhea vaccine**

2. Improving existing glycoconjugate vaccines

***Eg Streptococcus pneumoniae* (£2 billion per year)**

3. New markets

***Eg* Poultry and pig glycoconjugate vaccines**

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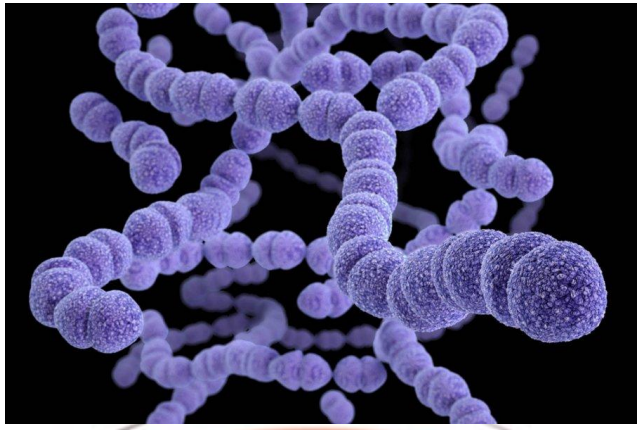
Streptococcus pneumoniae

Gram positive, alpha-haemolytic diplococcus

Over 90 different serotypes

Causes pneumonia, meningitis, conjunctivitis, bacteraemia and otitis media

Estimated that globally one million children under five die of pneumococcal disease each year



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Streptococcus pneumoniae Glycobod team

How can vaccine potency be improved?

How to upscale and manufacture a Strep pnuemo glycoconjugate?

Develop the E. coli glycocell to express diverse glycan?

What makes a good glycoconjugate vaccine?

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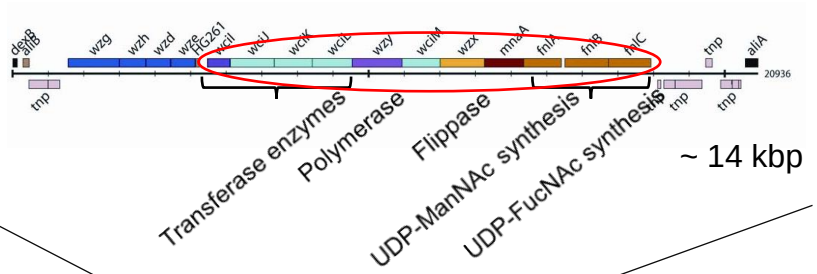
Strep pneumoniae vaccine design – general strategy



Capsules 4, 5, 8, 12F

Glycan synthesis genes

Cj PglB

Coupling enzyme

Capsules predicted to be CjPglB compatible:
1, 4, 5, 12F, 12A, 12B, 25F, 25A, 38, 44, 45, 46

Carrier proteins

D/E-Y-N-X-S/T

Acceptor protein

Genetically engineered *E. coli* host (mini factory)

Affinity purification

Glycoconjugate vaccines

First generation:
- Cj AcrA
- Pa ExoA

Second generation:
- Strep pneumo proteins (double hit vaccines)

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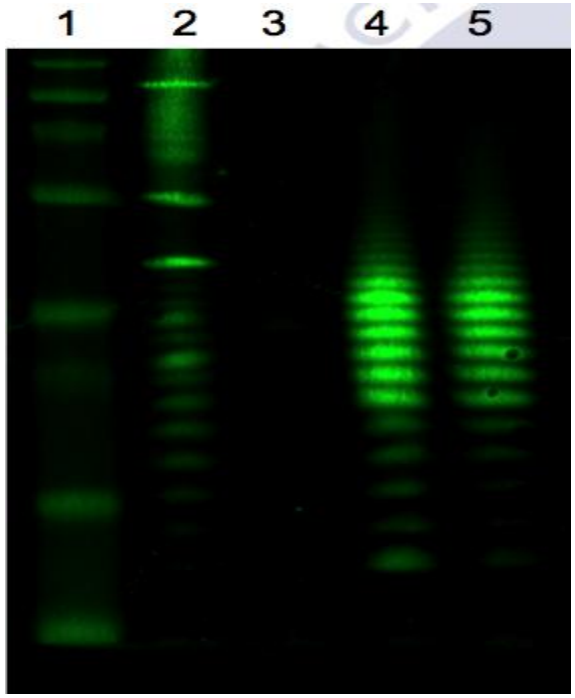
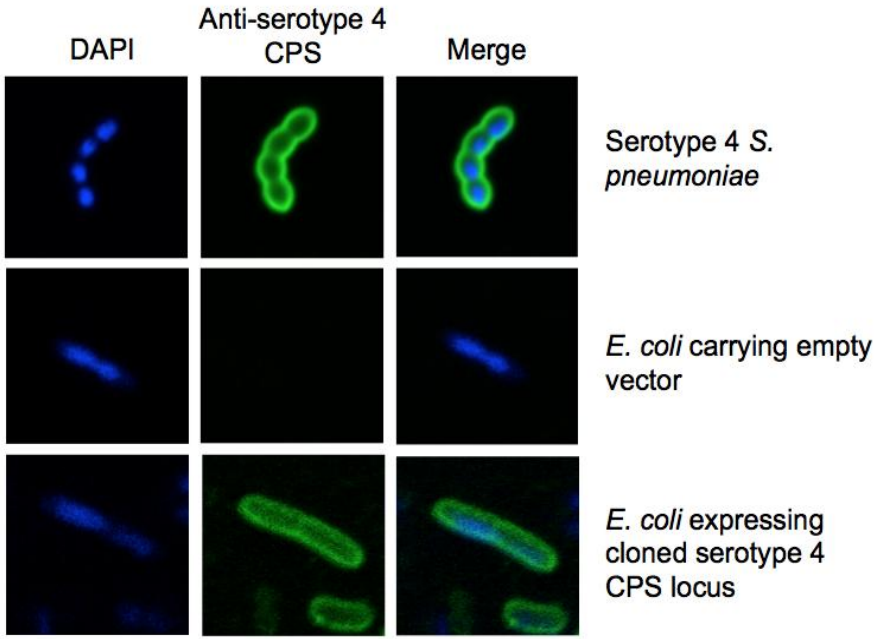
Strep pneumoniae capsule expressed in *E. coli*



Type 4, 8, 12F, 38 & 46 capsules expressed in *E. coli*



Confirmed cell surface expression

Kay *et al* Open Biol 2016

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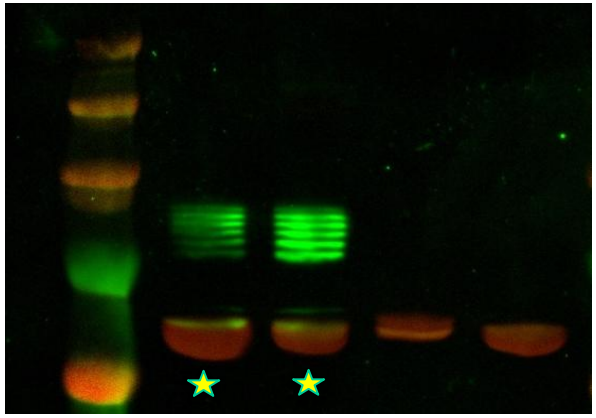
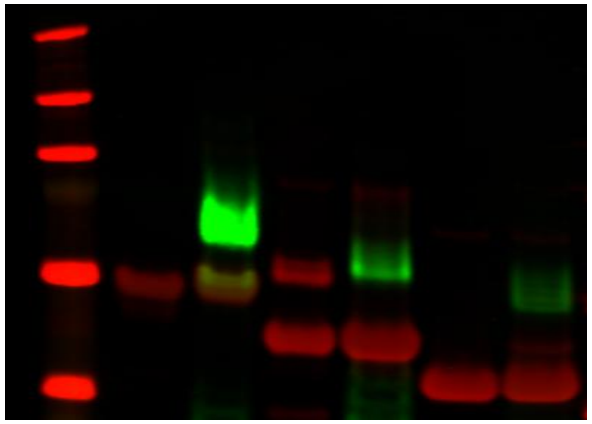
Double-hit glycoconjugate vaccines coupling *S. pneumo* capsules to *S. pneumo* proteins



Modification of the *S. pneumo* acceptor protein pneumolysin with serotype 4 capsule (stars). Green bands are glycoconjugate vaccine.



Three further recombinant glycoconjugate vaccines (green bands) with *S. pneumo* acceptor proteins x, y and z coupled to serotype 4 capsule.

Herbert *et al* Vaccine 2018
Reglinski *et al* Nature Vaccines 2018

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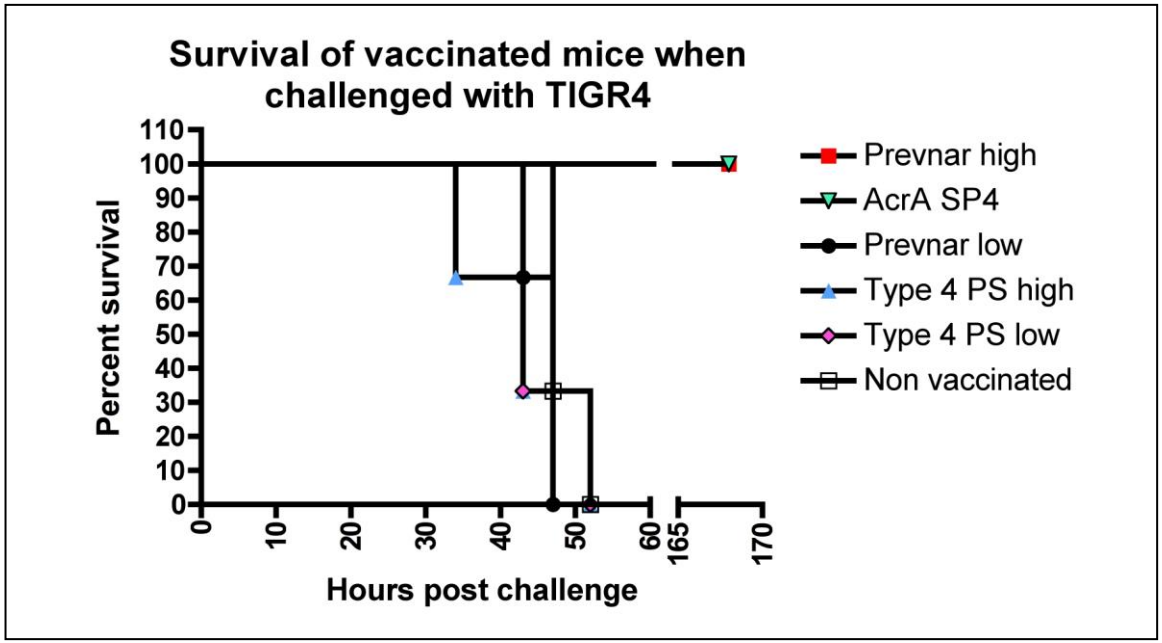
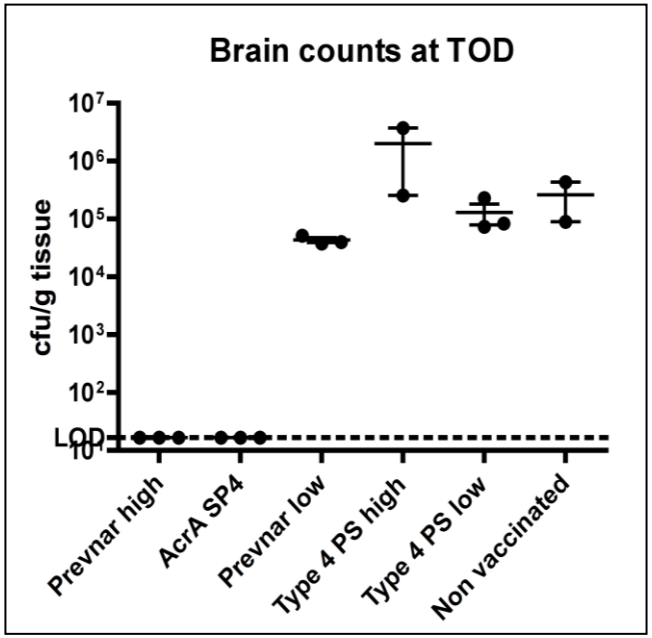
S. pneumoniae SP4 capsule coupled to AcrA alone protects and is as good as commercial vaccine





Herbert *et al* Vaccine 2018
Reglinski *et al* Nature Vaccines 2018

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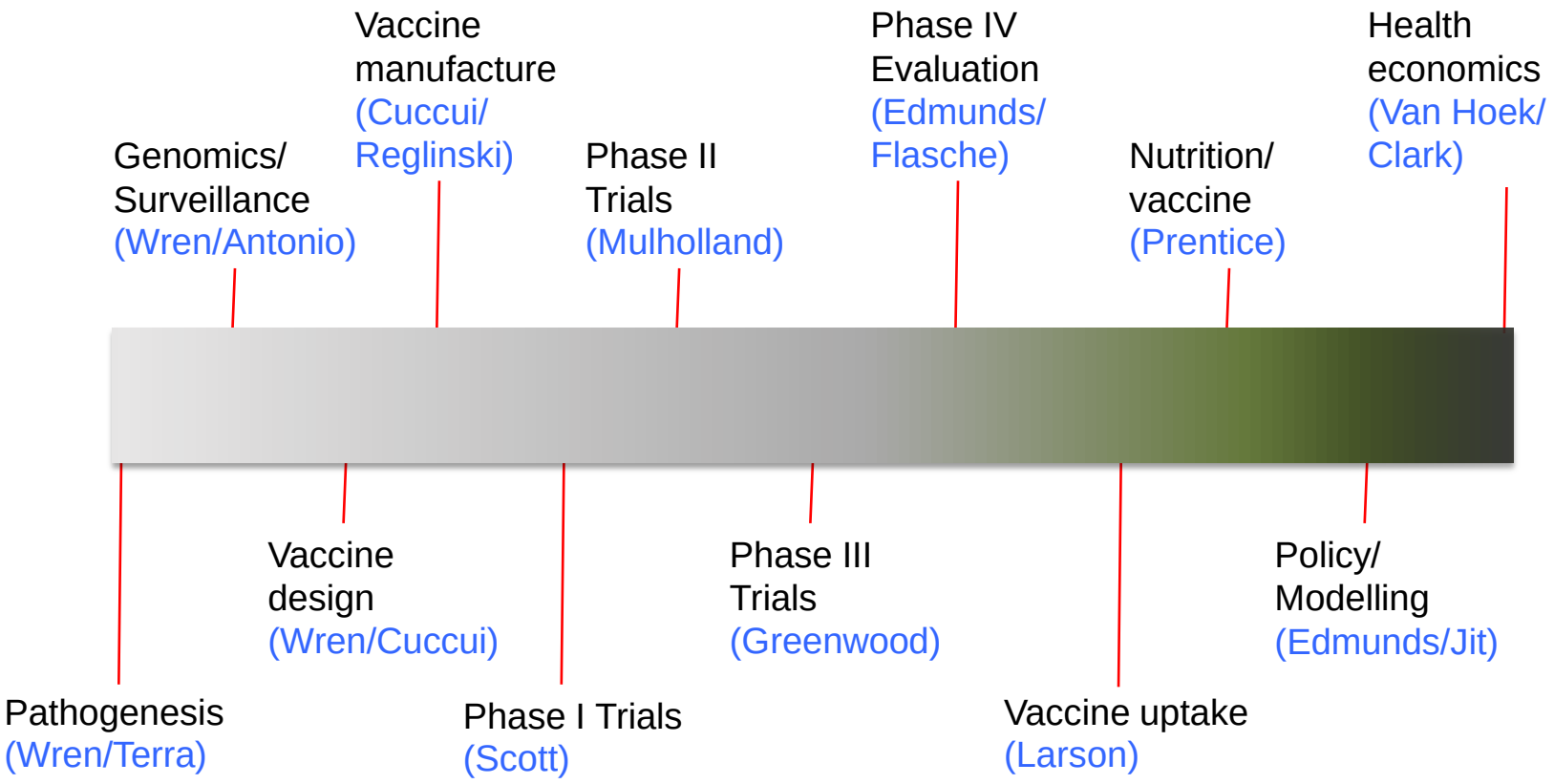
Why at LSHTM? – holistic approach to pneumococcal vaccine research and evaluation



Bench to Bush to Bedside

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Current recombinant glycoconjugate vaccines

1. New vaccines

***Eg Francisella tularensis, Burkholderia pseudomallei, Coxiella burnetii, Clostridium difficile, Brucella species, Shigella species* and Traveller's diarrhea vaccine**

2. Improving existing glycoconjugate vaccines

***Eg Streptococcus pneumoniae* (£2 billion per year)**

3. New markets

***Eg* Poultry and pig glycoconjugate vaccines**

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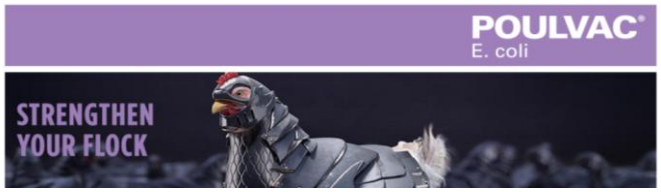
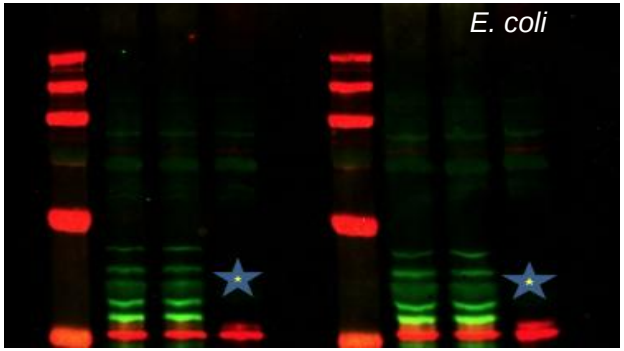
Glycoengineering for veterinary vaccines

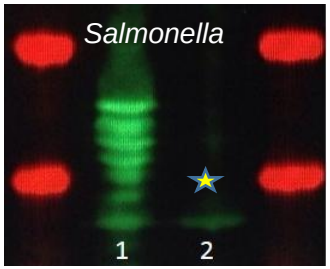
1. Triple poultry vaccine – *Campy* glycan coupled to *perfringens* protein in attenuated *E. coli* or *Salmonella* strain



2. Dual pig vaccine – *Strep suis* capsule coupled to App toxin

3. Dual bovine vaccine – *Coxiella* O-antigen coupled to *perfringens* protein





Recent BBSRC £5 million multicentre grant

LSHTM spin out – ArcVax (animal vaccines)

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Other PGCT (bioconjugate) bacterial vaccines

GSK buys vaccine specialist GlycoVaxyn for $190m

Acquisition will expand GSK's early vaccines pipeline

Article by
Phil Taylor

11th February 2015

From: Sales

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GlaxoSmithKline (GSK) has taken control of Swiss company GlycoVaxyn in a $190m deal that bolsters its early vaccines pipeline.

GSK already owned a stake in GlycoVaxyn and had been working with the company since 2012 on the development of conjugate vaccines for bacterial infections.

Tags

GSK
GlycoVaxyn
pharma takeover

1. **MRSA glycan/ExoA – protective in mice (Glycovaxyn) (JID 2014)**

2. **Uropathogenic *E. coli* – human trials (Glycovaxyn, Zurich)**

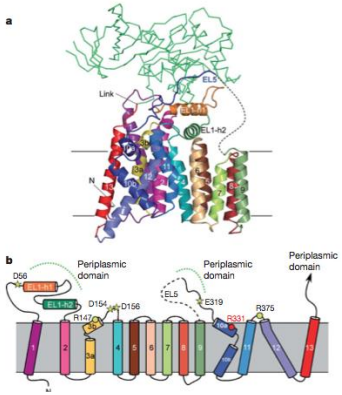
3. ***Shigella* LOS/ExoA – human trials (Glycovaxyn, Zurich)**

Limitations of PGCT based on *Campylobacter jejuni* PglB

- **The CjPglB enzyme only accepts acetylated reducing end sugars**
- **β1→4 linkages at penultimate sugar are not permissive**
- **PglB functions in the periplasm and is unlikely to function in Gram positive organisms**
- **Only glycans assembled on UndPP are transferred to an acceptor protein.**

Overcoming limitations

- **Mining for alternative PglBs from other bacteria in the hope that a variant exists with more relaxed sugar substrate specificity eg deep sea vent NtPglB (Mills et al., Glycobiol 2015).**
- **Directed evolution targeting specific regions of structurally characterised PglB (eg typhi vaccine).**
- ***O*-linked system (PglL) can accept any reducing end sugar, however, a true sequon for glycosylation has not been elucidated**
- **Alternative glycosylation systems**



Lizak *et al.* Nature 2011

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Bacterial glycosylation – coming of age

Four general classes of bacterial glycosylation systems

1) *O*-linked OTase-dependent, the glycan is assembled onto a lipid and then transferred to acceptor proteins in the periplasm by the Otase (*Neisseria*).

2) *O*-linked OTase-independent, sugars are individually added to target proteins by glycosyltransferases in the cytoplasm (*Campylobacter* flagellin modification).

3) *N*-linked OTase-dependent, the glycan is assembled onto a lipid and then transferred to acceptor proteins in the periplasm (*Campylobacter* general glycosylation system).

4) *N*-linked OTase-independent, the glycan is assembled in the cytoplasm (*Haemophilus infuenzae*).

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Current glycosylation studies on pet pathogens

Clostridium difficile **- both flagellin and S-layer are glycosylated**
Faulds-Pain *et al* Mol Micro 2014, Valiente *et al* J Biol Chem 2016
Bouche *et al* J Biol Chem 2016, Richards *et al* J Biol Chem 2018

Burkholderia **glycosylation x 2**
PglL mutant highly attenuated

Vibrio cholerae **glycosylation**

Francisella **glycosylation x 2**

Actinobacillus **glycosylation (new *N*-linked system)**
Cuccui *et al* Open Biology 2017

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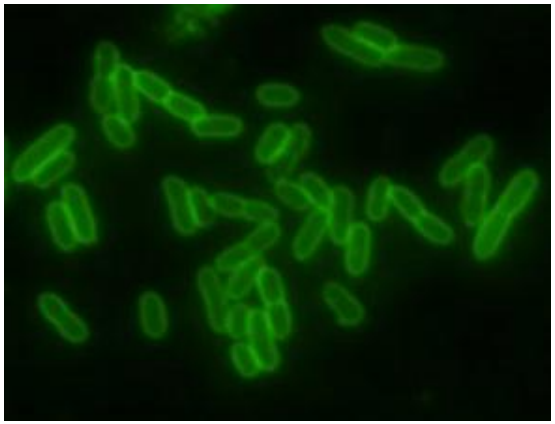
Glycobiology and glycoengineering

Academic pursuit



Translational product

Why glycosylate?

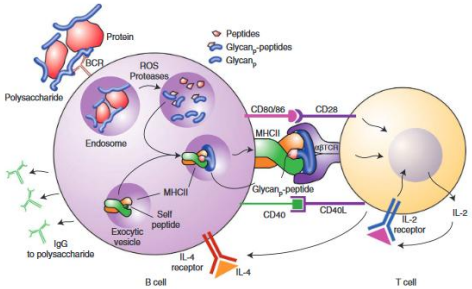
Role in patho genesis?

New systems?

Multiple vaccine candidates?

What is the best carrier protein? Why CRM197?

What makes a good glycoconjugate vaccine?



Avci et al., Nat Medicine, 2011

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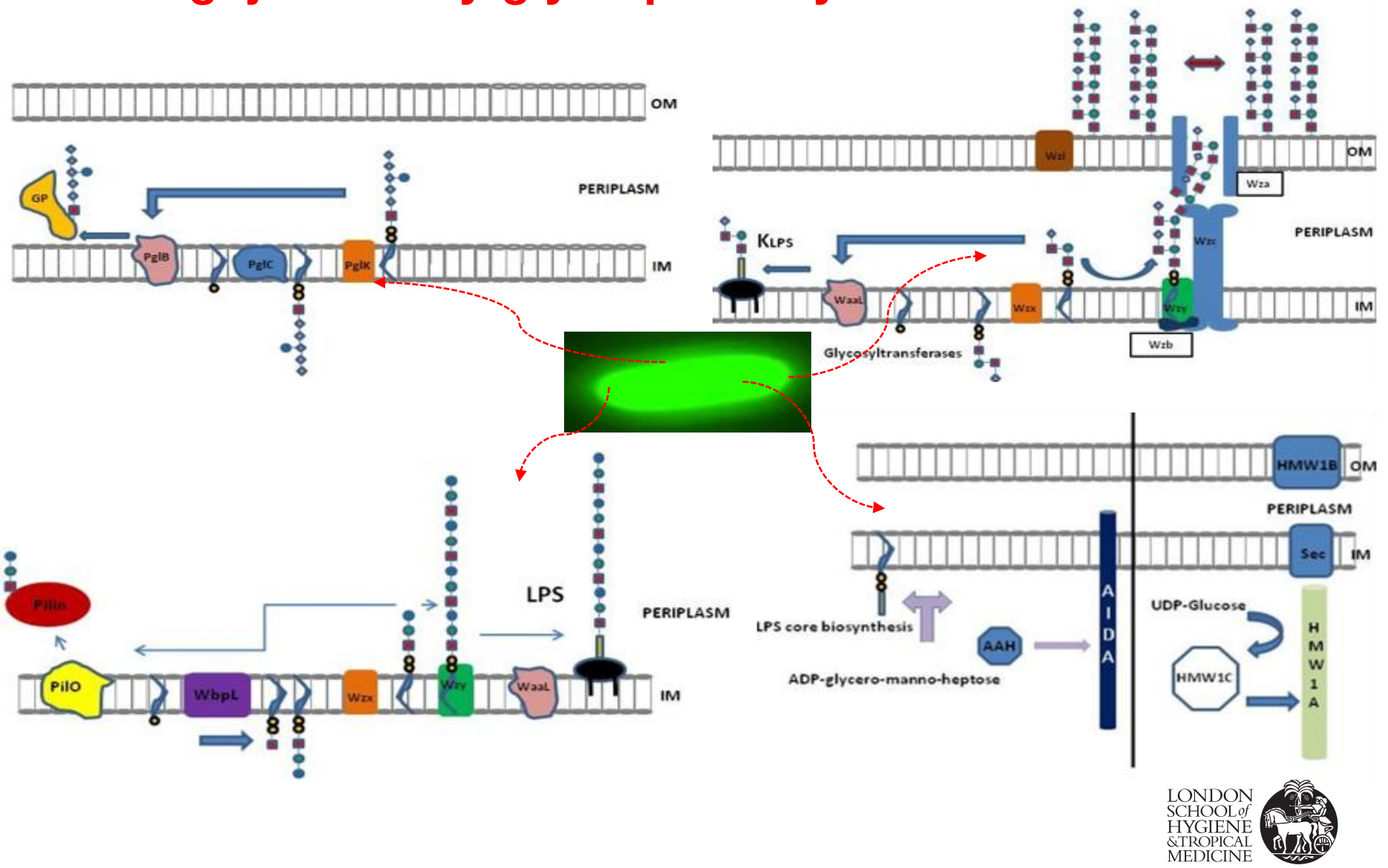
Beyond vaccines - bacteria are the best glycoengineers, can highjack many glyco pathways



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Conclusions and future perspectives

Basic curiosity driven research can lead to practical applications

1. **Inexpensive**
2. **In-exhaustible and homogeneous supply of vaccine**
3. **Versatile technology - coupling glycans with carrier proteins**
4. **Preserves antigen structure**
5. **“Double-hit” vaccines (*eg S. pneumo* protein with *S. pneumo* capsule)**
6. **Piggy back onto existing attenuated vaccines for multiple protection**
7. **Animal vaccines, not just for animal health & economic prosperity, but blocking zoonotic infections reduces human disease (One Health)**
8. **Better vaccines (humans and animals), less antibiotic use**

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Acknowledgements – LSHTM & PGCT

Current LSHTM
Sherif Aboelhadid
Lizzie Atkins
Michelle Cairns
Jon Cuccui
Lisa Dawson
Elizabeth Donahue
Nick Dorrell
Jennifer Dow
Abdi Elmi
Alex Faulds-Pain
Alex Shaw
Ozan Gundogdu
Emily Kay
Marta Mauri
Ian Passmore
Tim Scott
Richard Stabler
Vanessa Terra
Sam Willcocks

Past LSHTM
Elaine Allan
Suaad Al-Jaberi
Olivia Champion
Stewart Hinchliffe
Sarah Howard
Adrian Jervis
Andrey Karlyshev
Rebecca Langdon
Jiali Lim
Dennis Linton
Eric Sampane Donker
Melissa Martin
Dominic Mills
Madeleine Moule
Nevida Nez
Pippa Strong
Gill Thacker
Kerstin Williams

Acknowledgements - External

PGCT

Dennis Linton & Adrian Jervis (Manchester)

Francisella

Jo Prior (DSTL)

Strep pneumoniae

Tim Mitchell (Birmingham) & Jerry Brown (UCL)

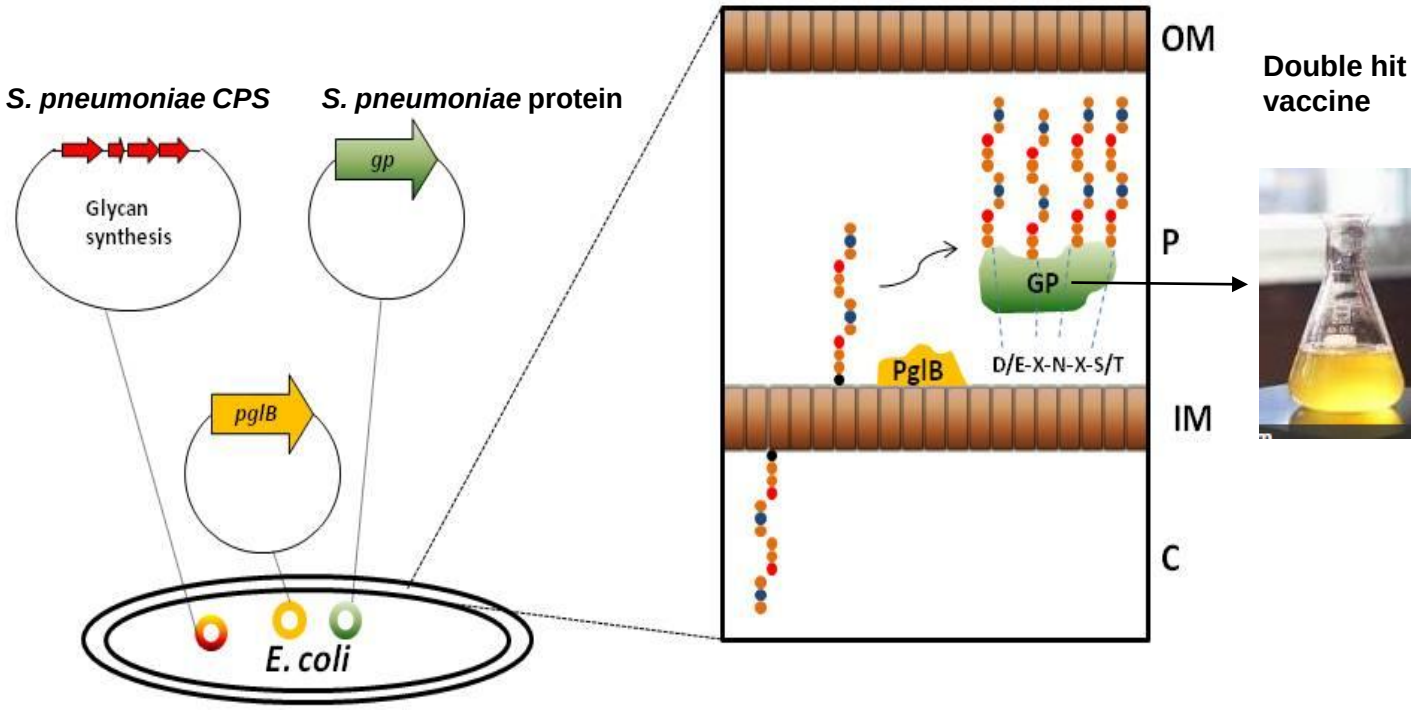
***N*-linked glycosylation discovery**

Markus Aebi (ETZ), Michael Wacker (Glycovaxyn), Christine Szymanski & Mario Feldman (UoA)

FUNDING


Thank You!!

Production of double-hit recombinant pneumococcal glycoconjugate vaccine



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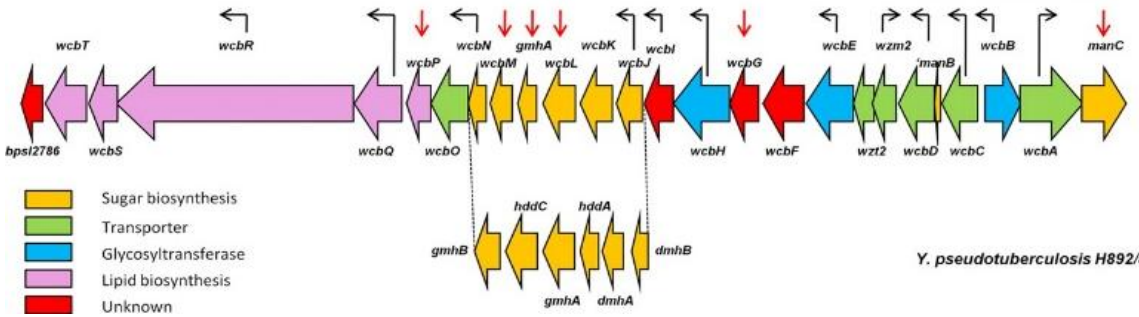
TECHNOLOGY

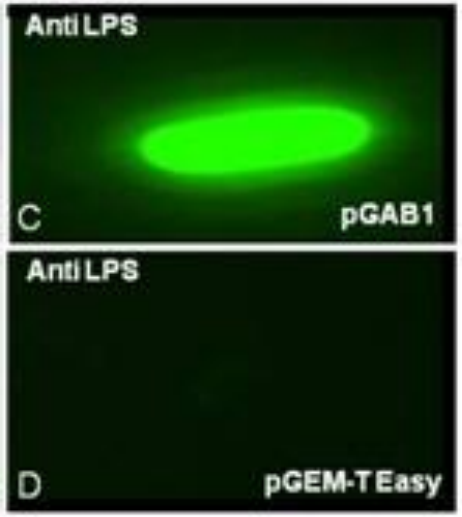


Step 1: Genetic information responsible for generating a sugar structure is cloned and transferred into a safe laboratory strain of *E. coli*

Step 2: Express foreign sugar structure
GLYCAN EXPRESSION TECHNOLOGY

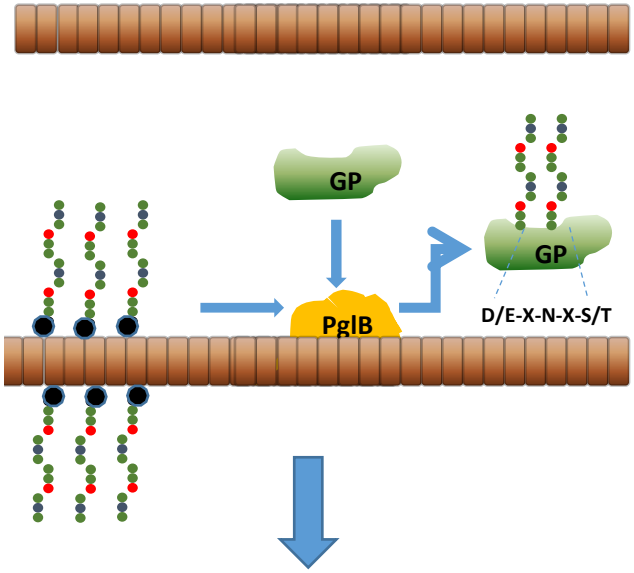
Engineered bacterial strain

Control bacterial strain



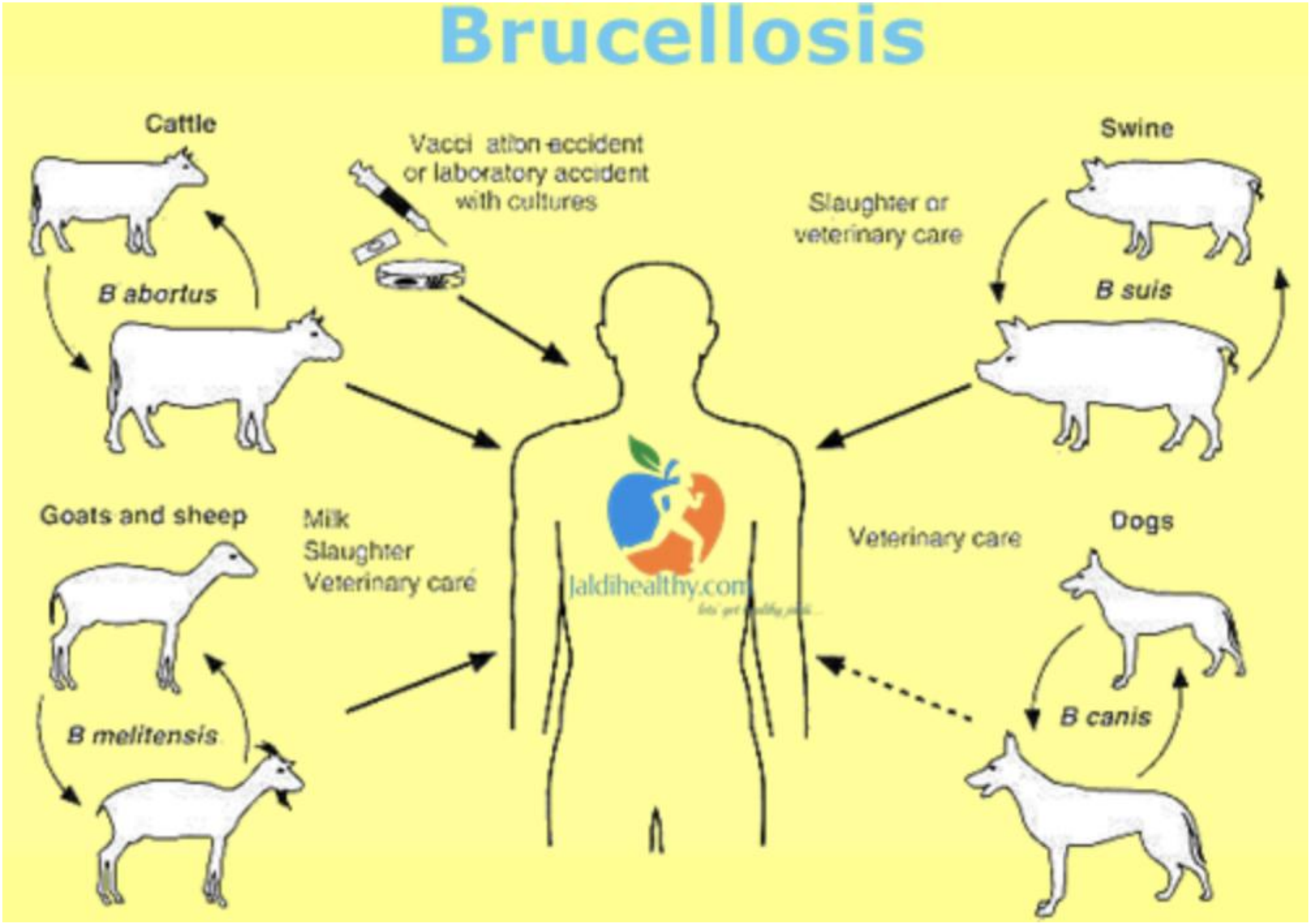
Green glow demonstrates coating of the cell with the new sugar structure

Step 3: Add genetic information for the coupling enzyme PglB and the acceptor protein
PROTEIN GLYCAN COUPLING TECHNOLOGY



Step 4: Purify newly generated glycoconjugate for vaccine testing

Brucellosis



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Yersinia/Brucella dual vaccine

- ***Yersinia enterocolitica***
  - causes yersiniosis, an animal-borne disease occurring in humans, as well as cattle, deer, pigs, and birds.
  - the portal of entry is the gastrointestinal tract normally *via* insufficiently cooked or contaminated water, meat, or milk.

- ***Brucella melitensis***
  - It can infect sheep, cattle, and sometimes humans
  - It is zoonotic causing Malta fever or localized brucellosis in humans

- ***Brucella abortus***
  - found in cattle populations; a blood borne pathogen that causes premature abortion of a cattle fetus
  - in humans this disease cause both acute and chronic symptoms.

Current vaccine

- Attenuated *Brucella abortus* strains
- Still virulent in humans
- Cannot discriminate between vaccinated and unvaccinated animals

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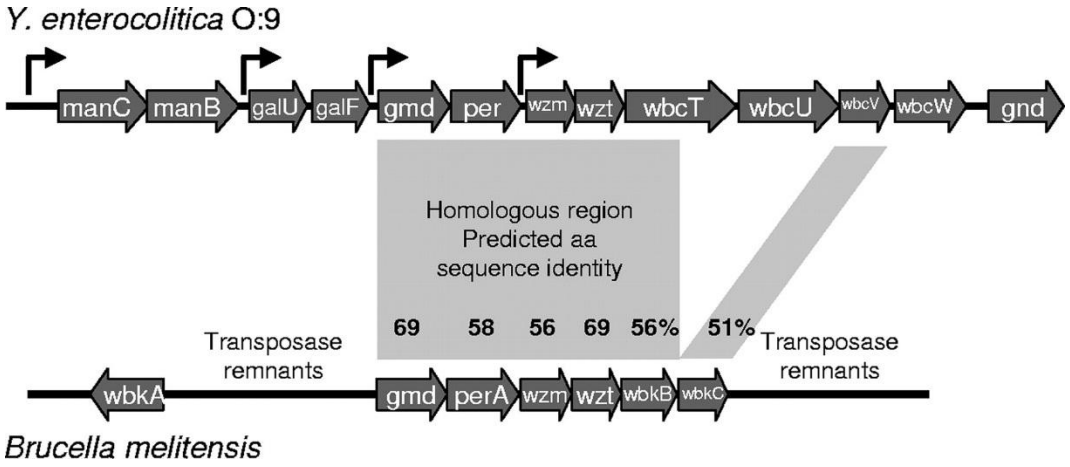
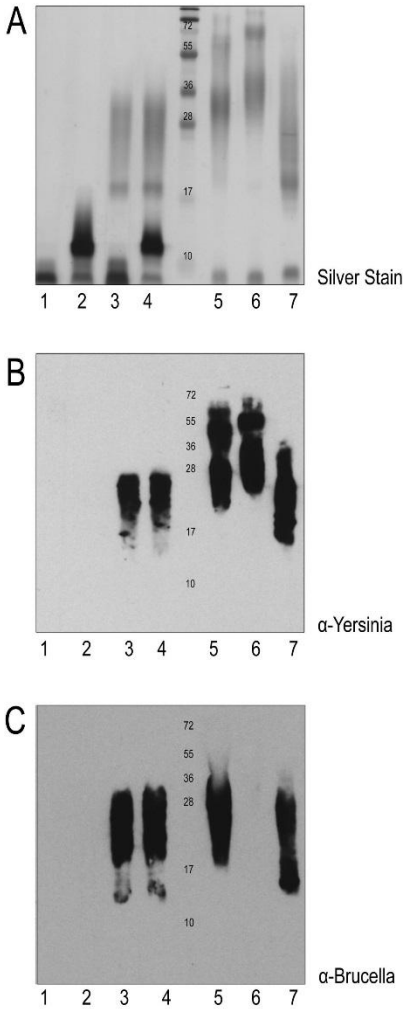
Yersinia/Brucella glycan identical

- Yersinia O9 and *Brucella melitensis* has the same A epitope
  - Homopolymer 1,2 linked 4,6-dideoxy-4-formamido-a-D-mannopyranosyl (N-formylperosamine)





Therefore, can highjack the O-antigen pathway from *Yersinia* by glycoengineering directly in a *Yersinia* O9 strain

Iwashkiw, 2012

Double-hit *Brucella* vaccine with the Omp25 antigen

Omp25 designed with glycotags, leader sequence, histag, restriction sites and then cloned into pUC19

EcoRI pelb leader glycotag histag BamHI

Gcgcgaattcgggatgaaatacttattgccgaccgctgcagccgggttgttattattagccgcacaaccc
gcaatggctatggatcaaaacgcaacggccgacgccatccaggaacagcctccggttccggctccggttg
aagtagctccccagtatagctgggctggtggctataccggtctttaccttggctacggctggaacaaggc
caagaccagcaccgttggcagcatcaagcctgacgattggaaggctggcgcctttgctggctggaacttc
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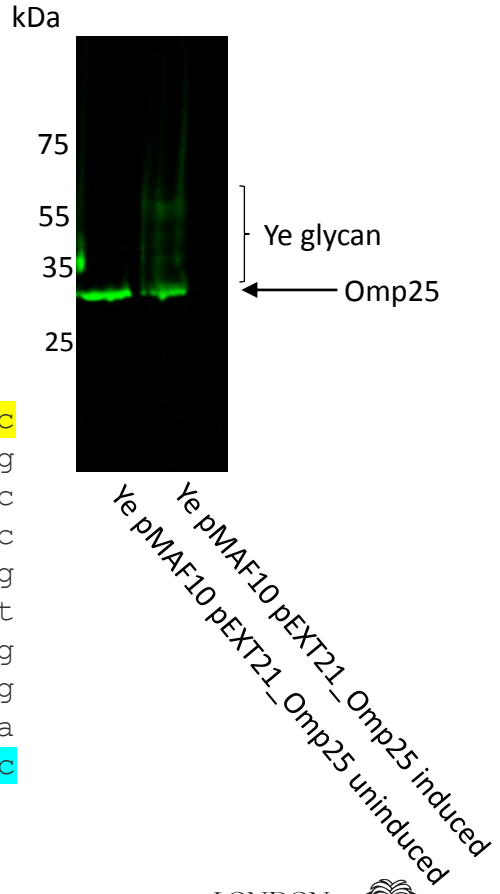

Excellent double-hit *Brucella* glycoconjugate vaccine

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Brucella vaccine - next steps

- **Put PglB on *Yersinia* chromosome to improve glycosylation**
- **Optimise purification of glycosylated Omp25**
- **Find collaborator to test vaccine candidates**
- **Couple with immunogenic *Coxiella* protein CBUxxxxx to make Brucellosis/Q fever dual vaccine**

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Summary and future plans

Curiosity driven research can lead to practical applications

- **The development of Glycan Expression Technology and Protein Glycan Coupling Technology**
- **Application to the production of novel inexpensive glycoconjugate vaccines**
- **Discovery of other bacterial glycosylation systems, role in pathogenesis and potential exploitation**
- **Long term plans – standard production of glycoproteins in *E. coli* - a new era in glycobiotechnology**

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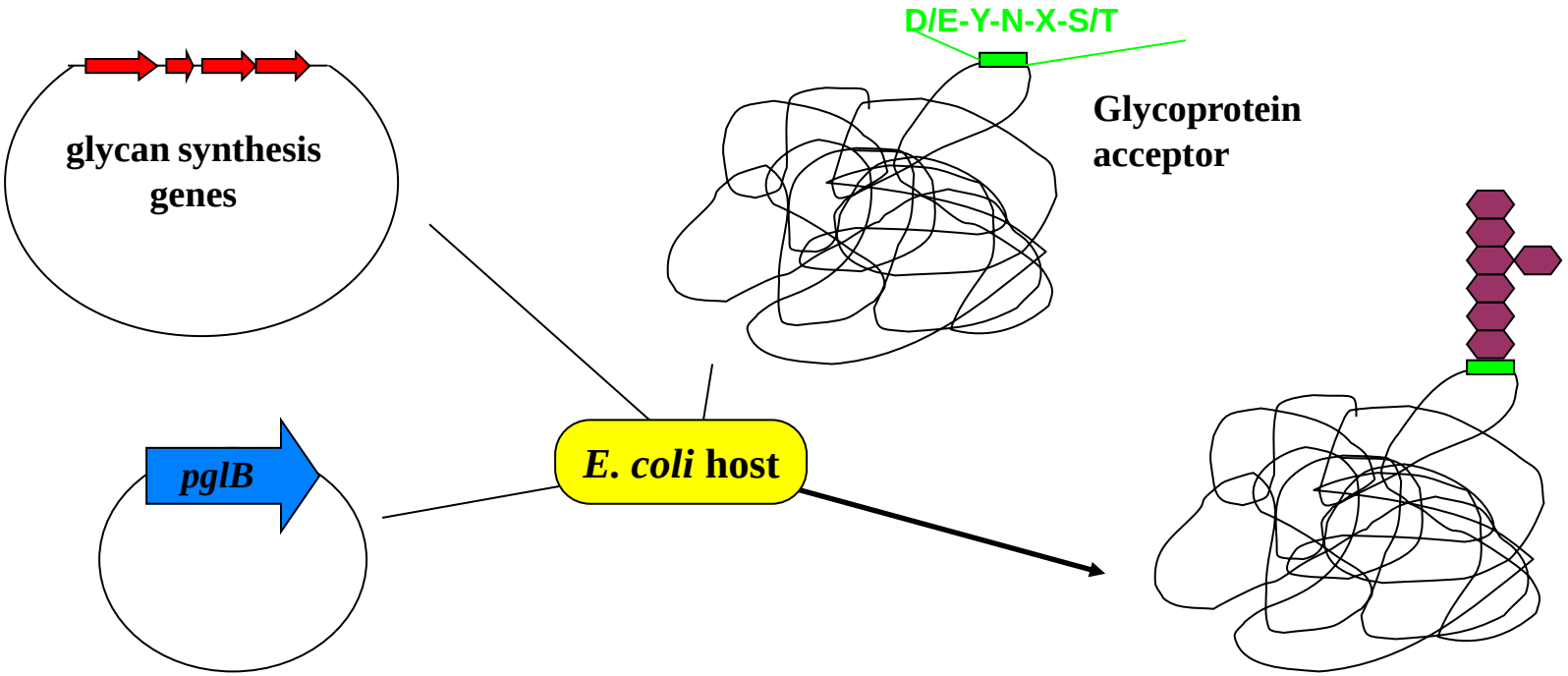
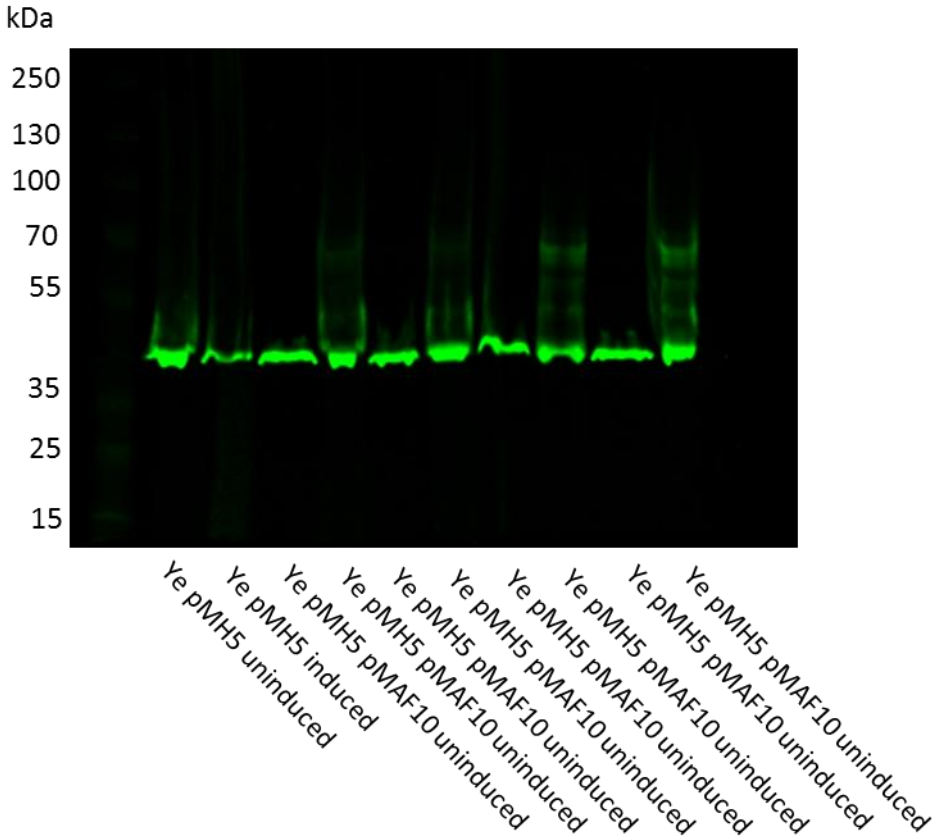
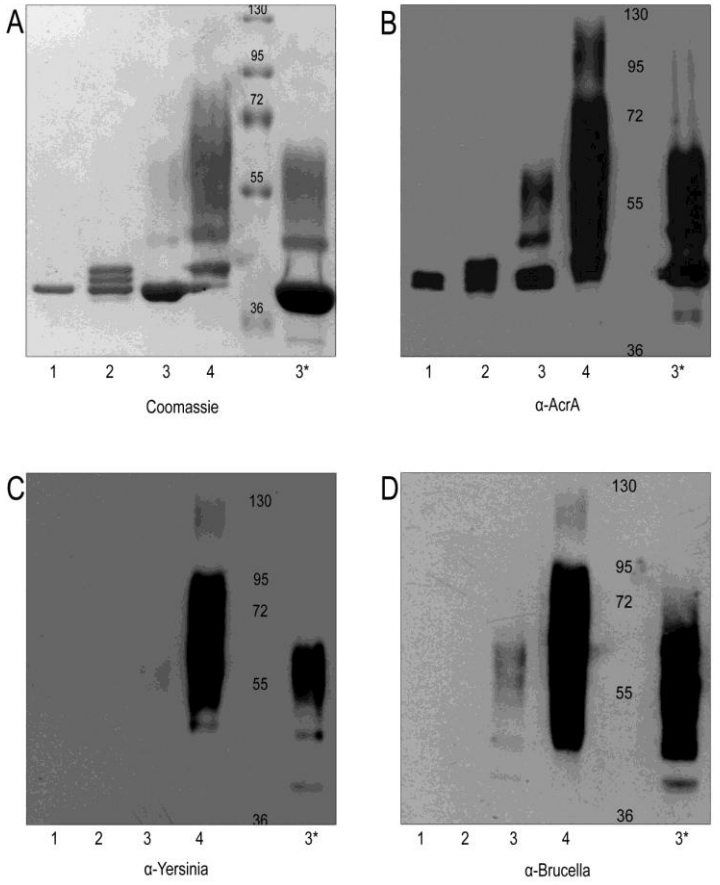
Protein Glycan Coupling Technology



In built flexibility should allow mixing & matching of protien/glycan combinations

PglB attaches glycan to AcrA





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